The Journal of Laboratory and Clinical Medicine

VOL. V. ST. LOUIS, MARCH, 1920 No. 6

ORIGINAL ARTICLES

CHEMICAL CHANGES IN THE BLOOD IN DISEASE*

BY VICTOR C. MYERS, PH.D., NEW YORK CITY

INTRODUCTION

ACCURATE data on the chemical composition of the blood, especially of the nonprotein fraction, are of comparatively recent origin, and are primarily the result of American observations with American methods. We owe largely to Folin, Benedict, and Van Slyke the development of the methods which have made this work possible. The practical information which these methods have made available has been especially helpful, since they have given us very valuable data on just those conditions on which the older methods of blood examination, cytology, bacteriology, serology, gave little information—reference is made principally to such constitutional conditions as nephritis, diabetes, and gout.

Although it is the primary object of the present paper to consider those observations which have served as a guide to the diagnosis and treatment of disease, it may be well at the outset to indicate the rather broad scope which chemical methods have recently taken in the study of the blood. They have included methods for the blood volume, the blood proteins, serum albumin and globulin, improved methods of hemoglobin estimation; methods for the determination of the nonprotein nitrogen and its individual components, urea, creatinine, uric acid, amino acids, creatine and ammonia; methods for sugar, and for the lipoid constituents, fat, lecithin and cholesterol; methods for the mineral constituents, chlorides, phosphates, calcium, magnesium, potassium, sodium and iron; methods for the blood gases, carbon dioxide, carbon monoxide and oxygen, and methods for the hydrogen ion, the acetone bodies, phenol, and such enzymes as diastase, catylase, etc.

*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.

This is the introductory part of a paper read in Symposiums on the Clinical Examination of the Blood before the New York Academy of Medicine on April 17, 1919, and the Cleveland Academy of Medicine on October 17, 1919. The present paper is the first of a series of papers to appear on this topic.

TABLE I

SIGNIFICANT CHEMICAL CHANGES IN THE BLOOD IN DISEASE*

CONDITION	UREA N	SUGAR	CO_2-COMBINING POWER	CREATININE	URIC ACID	NON-PROTEIN N	CHOLESTEROL	CHLORIDES AS NaCl	DIASTATIC ACTIVITY
	mg. to 100	per cent	c.c. to 100	mg. to 100	mg. to 100	mg. to 100	per cent	per cent	
1. Normal	12–15	0.09–0.12	50–75	1–2	2–3	25–30	0.14–0.17	0.57–0.62	15–20
2. Beginning pathologic	20	0.15	–45	3.5	4	35	0.19	{ –0.55 / +0.65	25
3. Renal diabetes		0.08–0.12							
4. Mild diabetes		0.15–0.30							25–40
5. Severe diabetes	20	0.30–1.20	50–10	2–4	4–10		0.2–0.8	0.5	35–75
6. Gout					4–10				
7. Early interstitial nephritis	15–25	0.12–0.15		2–3.5	5–12				
8. Acute nephritis	40–100	0.12–0.18	45–20	2–6	5–15				
9. Parenchymatous nephritis (nephrosis)	20–50	0.12–0.20		2–4	2–5		high	to 0.75	
10. Terminal interstitial nephritis	60–300	0.12–0.24	40–12	5–28	5–27	100–350	to 0.30	to 0.46	20–50
11. Bichloride poisoning, to	300	0.12–0.20		33	15	370	0.35	0.5–06	
12. Double polycystic kidney, to	75	0.20		8	5				
13. Prostatic obstruction	12–40	0.11–0.16		1.5–3.5	3–9				
14. Acute intestinal obstruction	45–120					75–170			
15. Eclampsia	10–25		58–43			25–45			
16. Cholelithiasis							0.13–0.30		
17. Pernicious anemia							to 0.06		

*The data recorded in Table I are from our own observations except the figures under numbers 14, 15 and 16. The data on intestinal obstruction were taken from Tileston and Comfort,[1] on eclampsia from Losee and Van Slyke[2] and on cholelithiasis from Rothschild and Rosenthal.[3] The figures under 11 and 12 (to) simply indicate the maximal values we have observed in these conditions.

As already pointed out, these methods have yielded especially helpful information in diabetes, nephritis, and gout, while the data obtained in renal diabetes, infantile conditions such as tetany and the diarrheal acidoses, in eclampsia, malignancy, cholelithiasis, pernicious anemia, disorders of the ductless glands and various urologic conditions, have given us a new point of view regarding many of these disorders.

Before proceeding to a discussion of the pathologic variations in the composition of the blood, it may be well to indicate in tabular form the range of changes usually encountered in several common metabolic disorders. From an inspection of Table I it will be apparent that the tests furnish information of great value in diagnosis, prognosis, and treatment. Data are given on nine different blood constituents, the first five of which are regarded of special significance, and are arranged in order of their supposed clinical importance; viz., urea, sugar, CO_2-combining power, creatinine and uric acid.

A discussion of the various conditions will be taken up in the order in which they are presented in Table I. It is difficult to draw an arbitrary line indicating where normal findings end and pathologic begin, but it is believed safe, when the blood is taken after a 14-hour fast (in the morning before breakfast), to regard a urea nitrogen above 20 mg. and a sugar above 0.15 per cent as quite definitely pathologic, etc.

RENAL DIABETES

It will at once be noted in the case of renal diabetes that the blood sugar is perfectly normal, and it is only when a knowledge of this fact is at hand that a definite diagnosis of this rather uncommon condition can be made. Here the threshold point of sugar excretion is below the level of the normal blood sugar. Broadly considered, renal diabetes may be regarded as a condition of glycosuria (glycuresis[4]) not dependent upon a temporary increase of blood sugar in an individual free from symptoms of diabetes mellitus. It should perhaps be noted that mild glycosuria occasionally appears to be associated with parenchymatous nephritis[5] in which case a slight hyperglycemia may be present, but quite without influence on the glycosuria.

DIABETES MELLITUS

In diabetes the examination is primarily directed to the determination of the sugar, although in advanced cases the acidosis, as indicated by the CO_2, may assume greater significance. Here the condition of lipemia may develop, and of this the cholesterol is a particularly good index.

It should be remembered that the excretion of sugar by the kidney is simply one of the body's many factors of safety, in fact it may be compared with the safety valve of a steam boiler. The real condition to which attention should be directed is the hyperglycemia, and as the disease advances the glycosuria becomes less and less a safe criterion of this, since the permeability of the kidneys for sugar appears to be gradually lowered. Normally the blood sugar varies from 0.09 to 0.12 per cent and in early cases of diabetes one may note an excretion of sugar when the sugar of the blood rises above 0.16 or 0.17 per cent, but in advanced cases showing nephritic symptoms, blood sugar figures of 0.2 to 0.3 per cent, and even more, may be noted without the appearance of any sugar in

the urine. We have observed that the diastatic activity[6] of the blood is proportional to the blood sugar in untreated cases and appears to be the blood sugar determinant. In those cases which develop a severe acidosis, the CO_2 determination of Van Slyke is a much more reliable and valuable index of the acidosis than any urinary determination. Any extended medical treatment or surgical interference should not be attempted on a severe diabetic without a knowledge of the blood sugar and the alkali reserve of the body as indicated by the CO_2 of the blood.

GOUT

The blood in gout is characterized by its rather high content of uric acid, figures of 4 to 10 mg. It should be borne in mind, however, that early cases of interstitial nephritis disclose similar figures, although here there is generally a tendency for urea retention as well. Since a purine-free diet lowers the blood uric acid, treated gout cases may occasionally be encountered where the uric acid is only slightly elevated. The estimation of the blood uric acid is a valuable aid to the diagnosis of gout, but only when considered in connection with the clinical symptoms and other laboratory findings.

NEPHRITIS

There would appear to be little doubt that early cases of chronic nephritis are accompanied by an appreciable rise in the blood uric acid, although a rise in the blood urea can probably be taken as a safer sign of impaired kidney function. It is certainly true that the urea nitrogen falls within very narrow limits for perfectly normal individuals. As soon as one passes to hospital patients, however, figures above 15 mg. of urea nitrogen are found. Figures over 20 on the usual restricted diet of the hospital would suggest impaired kidney function. Creatinine appears to be more readily eliminated than either uric acid or urea, and it is not, as a rule, until the blood urea has doubled, or more than doubled the normal, that there is a very appreciable increase in this purely endogenous waste product derived apparently from muscle metabolism. The normal for the creatinine of the blood is approximately 1 to 2 mg. per 100 c.c. and figures over 3.5 mg. can be viewed with grave concern, while figures over 5 mg. are almost invariably indicative of an early fatal termination.[8] The only possible exceptions are cases where the retention is due to some acute renal condition, such as acute nephritis and mild bichloride poisoning. How these changes gradually come about is well brought out in the accompanying staircase table (Table II).

The inability to properly excrete the waste products of nitrogenous metabolism is only one of the difficulties which arise as the result of renal disease. As is well known, in parenchymatous nephritis, or nephrosis, the edema is probably dependent, in part at least, on the lowered permeability of the kidney for chlorides with their consequent retention. It is natural, therefore, to expect that the excretion of other salts should be deficient, although we have only recently come to appreciate the effect of the retention of phosphates. Normally, acid phosphate provides one of the most important mechanisms of eliminating acid. When the phosphate excretion is impaired, bringing about an increase in the (acid) phosphate of the blood[9] (and tissues), an acidosis results, which may be quite as

TABLE II

STAIRCASE RETENTION OF URIC ACID, UREA AND CREATININE IN NEPHRITIS*

DATE 1915-16	CASE	AGE	SEX	DIAGNOSIS	CONDITION	MG. PER 100 C.C. OF BLOOD			PHTHALEIN 2 HRS. PER CENT.	SYSTOLIC BLOOD PRESSURE	URINE	
						URIC ACID	UREA N	CREATININE			ALBUMIN	CASTS
I												
9/17	H. L.	23	♂	Pulmonary tuberculosis	Unchanged	6.5	16	2.7	58	130	++	+
8/10	E. H.	41	♂	Pericarditis	Unchanged	5.6	13	2.1	45	150	−	−
10/12	F. D.	45	♂	Interstitial nephritis	Unchanged	5.5	12	2.5	37	185	−	+
3/ 6	B. D.	35	♀	Diffuse nephritis	Unchanged	9.6	19	2.4	45	175	+	+
II												
8/11	J. J.	65	♂	Early interstitial nephritis	Unchanged	9.5	25	2.5	13	185	+	+
7/21	D. S.	56	♂	Early interstitial nephritis	Unchanged	6.6	24	3.3	26	185	−	+
9/21	D. D.	52	♂	Early interstitial nephritis	Unchanged	8.7	20	3.6	20	100	+	+
8/ 3	C. M.	54	♂	Early interstitial nephritis	Unchanged	6.3	31	2.0	23	150	−	−
III												
1/ 6	L. P.	57	♂	Moderately severe chronic interstitial nephritis	Improved	8.0	80	4.8	0	240	++	++
3/ 1						4.9	17	2.9	10	170		
4/23	J. P.	34	♂	Moderately severe chronic diffuse nephritis	Improved	8.3	72	3.2	25	238	+++	++
5/21						5.3	21	1.9	43	145		
1/15	W. C.	49	♂	Moderately severe chronic diffuse nephritis	Improved	9.5	44	3.5	38	210	++	++
1/28						2.5	19	1.9	52	120		
IV												
4/11	E. C.	50	♀	Typical fatal case of chronic interstitial nephritis	Died	22.4	236	16.7	0	210	++	Pus
3/23	T. D.	34	♂	Typical fatal case of chronic interstitial nephritis	Died	15.0	240	20.5	2 - 3	225	++	+
1/25	S. H.	37	♂	Typical fatal case of chronic interstitial nephritis	Died	14.3	263	22.2	0	220	++	+
4/15	J. W.	34	♂	Typical fatal case of chronic interstitial nephritis	Died	8.7	144	11.0	Trace	225	+	+

*From Chace and Myers,[7] 1916.

severe as that resulting from diabetic ketosis, judging from the CO_2-combining power of the blood. All cases of advanced interstitial nephritis suffer from acidosis,[10] and in some cases this is apparently the actual cause of death. It may also be noted that cases of acute nephritis occasionally show marked acidosis. When this is relieved by the administration of alkali or otherwise, a simultaneous clinical improvement takes place.

In parenchymatous nephritis, if we may be allowed to use this term, the findings are quite different. Here the nitrogen retention is comparatively small, although, as noted above, the examination of the blood may disclose a retention of chlorides. The figures for urea nitrogen seldom exceed 30 mg. except in terminal stages of the disease, and generally fall between that figure and 15.

It is of interest in this connection to note that many advanced cases of malignancy,[11] possibly as a result of the toxemia, give the chemical blood picture of moderately severe nephritis, also that many cases of pneumonia show definite evidence of nitrogen retention, while in the terminal stages of the disease a severe acidosis quite generally develops.

It is apparent from the observations of Losee and Van Slyke[2] that eclampsia is accompanied neither by nitrogen retention nor severe acidosis. Although these observations are essentially negative, they are very helpful in that they show that this toxemia is not a "uremia."

These facts regarding the nitrogen retention and acidosis of nephritis are worthy of consideration from a surgical as well as from a medical point of view, especially when one considers that a very appreciable fall in the CO_2-combining power of the blood may result from the use of a general anesthetic such as ether. When the acidosis is already pronounced, it is easy to imagine what may happen from the additional fall of 10 to 15 volumes per cent, such a fall being not uncommon in individuals without acidosis as the result of ether anesthesia.

PROSTATIC OBSTRUCTION

The blood urea has been found to be a very valuable preoperative prognostic test in cases of prostatic obstruction.[12] Cases showing urea nitrogen figures under 20 mg. per 100 c.c. of blood may be regarded as good operative risks so far as the kidneys are concerned. When the urea nitrogen figures are found between 25 and 30, the patient should be operated on with considerable caution and best after a period of preliminary treatment directed to relieve the nitrogen retention. Urea nitrogen figures over 30 mg. are very good evidence of renal involvement, and therefore afford a rather poor operative prognosis.

CHOLELITHIASIS

Since gallstones are largely composed of cholesterol, it is reasonable to suppose that their appearance might be associated with an increase in the cholesterol content of the blood. Although it seems quite probable that a hypercholesterolemia is present during the early period of the formation of the calculi, analytical data show wide variations in the blood cholesterol,[3, 13] the findings ranging from low normals to figures that are definitely increased. This being the case, it is not possible to employ the cholesterol estimation in the blood as a satisfactory means of diagnosing cholelithiasis.

PERNICIOUS ANEMIA

It is of interest that in pernicious anemia the cholesterol content of the blood plasma is markedly decreased.[13] When the antihemolytic action of cholesterol is recalled, it will be seen that this observation may possess some practical significance. The therapeutic administration of cholesterol in this condition has received attention from Italian investigators and has apparently been followed by beneficial results.

MISCELLANEOUS CONDITIONS

Sufficient has been said to indicate the practical value of the chemical analysis of blood in pathologic conditions, especially those of a constitutional nature, although many others might be mentioned. In the diarrheal acidosis of infancy the CO_2-combining power has been very helpful in pointing the way to an accurate diagnosis and therapy. Disturbances of an endocrine origin have long been recognized to be associated with a change in carbohydrate tolerance. The estimation of the blood sugar after the administration of a suitable amount of carbohydrate has afforded a much more reliable method of bringing out deviations in tolerance than the older technic of examining the urine. In leucemia it is of interest that we frequently find a very marked increase in the uric acid content of the blood.

DIFFERENTIAL DIAGNOSIS

The physician encounters many conditions where a knowledge of the chemical blood findings is of great assistance in differential diagnosis. One of these has already been mentioned, viz., renal diabetes. Without a knowledge of the blood sugar content it is scarcely possible to suitably differentiate this condition from diabetes. The estimation of the blood uric acid is often of considerable assistance in the differential diagnosis of gout and arthritis, the uric acid being essentially normal in the latter condition. Cases are sometimes encountered which clinically might be diagnosed as cases of essential hypertonia without a knowledge of the chemical blood findings, but these disclose a high uric acid, definite urea retention and figures for creatinine somewhat higher than one would ordinarily expect from the urea, a picture which appears fairly characteristic of nephritis of the arteriosclerotic type. In essential hypertonia one may find a fairly marked increase in the uric acid, but obviously no urea or creatinine retention. Cases of apoplexy present some of the clinical symptoms of uremia, but negative data on the urea and creatinine of the blood definitely exclude "uremia" as the cause of these symptoms. Such cases should not be passed over, however, without a knowledge of the CO_2-combining power, as cases of acute nephritis are sometimes encountered without nitrogen retention but with pronounced acidosis.[10]

COLORIMETRIC METHODS

It is perhaps appropriate that some remarks be made regarding the very sensitive quantitative methods which have been developed for the chemical analysis of blood. The determination of the nine constitutents for which data are given in Table I may all be made colorimetrically except for the CO_2-combining power and chlorides. Such extensive use of colorimetric methods is a development of the past fifteen years. In 1904 Folin introduced his accurate and sur-

prisingly simple colorimetric method for the estimation of creatinine in the urine, and this gave a great impetus to the development of colorimetric methods. For this work Folin employed the Duboscq colorimeter and at the present time there are very few laboratories of physiologic chemistry that do not boast one or more of these instruments.

COLORIMETERS

Since the accuracy of colorimetric methods depends in considerable part on a satisfactory colorimeter, it may be of value to consider the mechanical principles upon which the several different instruments in common use are constructed. The instruments may be simply designated as the (1) plunger, (2) wedge or (3) dilution type. With the plunger type the intensity of the color of either the standard or unknown is varied by changing the depth of solution through which the light passes with the aid of a plunger. The Duboscq, Krüse, Kober and Bock-Benedict are of this type. With an instrument of the wedge type such as the Hellige, the standard is placed in a wedge and the unknown in a small cup with sides parallel to the wedge. The wedge is moved up and down until the intensity of the color is the same as the unknown. With the dilution type of instrument such as the Sahli hemoglobinometer and the inexpensive test tube instrument described by the writer, the unknown is diluted with water or some diluting fluid until it has identically the same color as the standard.

The instruments of the plunger type have been most extensively employed where accuracy and rapidity were required. Of the individual instruments on the market the Duboscq is the oldest and best known. Disadvantages of this instrument are the high cost and the fact that the cups and plungers are mounted in balsam and require frequent remounting. The original French instrument is very substantially constructed, however, and the glass parts are of excellent quality. This latter fact is very important, since good prisms are probably the most essential part of the instrument, otherwise it is not possible to make the two halves of the field appear the same. The Krüse model of the Duboscq, available before the war, contained some advantages over the French instrument, notably in the mounting of the cups and plungers, and was more reasonable in price, although in general it was hardly the equal of the Duboscq. The American-made Kober has many mechanical superiorities over the Duboscq and is less expensive. It has a much better arrangement for adjusting the stages than the old style rack and pinion, the vernier scale may be readily set at zero, the reflecting mirrors are divided, while the cups and plungers are of black glass with fused-on clear glass bottoms, thus making the field considerably sharper. With these cups, chloroform may be used with impunity. It should be noted, however, that the cups are much more fragile than in the Duboscq, and it is doubtful whether the prisms are as evenly matched as in the French instrument. A very simple and relatively inexpensive colorimeter has recently been introduced by Bock and Benedict.[14] In this instrument small reflecting mirrors have been substituted for the expensive prisms. The standard is put in a cell of known breadth through which the rays of light pass longitudinally, thus removing the necessity for more than one moving part. Colorimeter estimations may be made very rapidly with the instrument, and it is possible, when the instrument is precisely constructed to make exceedingly accurate colorimetric comparisons, owing probably to the fact that the glass prisms have been eliminated. Considerable personal experience with all four instruments mentioned has led the writer to choose this instrument for routine work.

The Hellige instrument has the advantage that permanent standards may be kept in individual wedges, but the disadvantages that few of these standards are really permanent and that the wedges need to be empirically standardized. When a wedge is carefully calibrated for a given purpose by the individual using the instrument, very satisfactory results may be obtained. However, just the individuals to whom this instrument especially appeals are the ones who will not insure the accurate calibration of the standard wedges.

With the dilution type of instrument such as the Sahli hemoglobinometer and the inexpensive test tube instrument described by the writer,*[15] the unknown is diluted with water

*An improved model of this instrument is now sold by the C. M. Sorensen Co., Inc., New York City, who also distribute the Bock-Benedict and Kober colorimeters.

or some diluting fluid until it has identically the same color as the standard. Theoretically, the principle of diluting the unknown to the same intensity of color as the standard is excellent, but practically, it requires considerable care in execution and fairly large volumes of fluid in order to secure accuracy, since the diluting fluid must be added a drop or a few drops at a time. Where the volume of fluid is large enough that one or two drops of fluid more or less do not impair the accuracy of the test, as in the test tube instrument mentioned above, the method is perfectly satisfactory, especially for an occasional estimation, but in such very small instruments as the Sahli and Kuttner a large error is necessarily introduced.

REFERENCES

[1]Tileston and Comfort: Arch. Int. Med., 1914, xiv, 620.
[2]Losee and Van Slyke: Am. Jour. Med. Sc., 1917, cliii, 94.
[3]Rothschild and Rosenthal: Am. Jour. Med. Sc., 1916, clii, 394.
[4]Benedict and Osterberg: Jour. Biol. Chem., 1918, xxxiv, 258.
[5]Myers and Kast: Jour. Biol. Chem., 1920, xli (proceedings).
[6]Myers and Killian: Jour. Biol. Chem., 1917, xxix, 179.
[7]Chace and Myers: Jour. Am. Med. Assn., 1916, lxvii, 929.
[8]Myers and Killian: Am. Jour. Med. Sc., 1919, clvii, 674.
[9]Marriott and Howland: Arch. Int. Med., 1916, xviii, 708.
[10]Chace and Myers: Jour. Am. Med. Assn., 1920, lxxiv, 641.
[11]Kast and Killian: Proc. Soc. Exper. Biol. and Med., 1919, xvi, 141.
[12]Squier and Myers: Jour. Urol., 1918, ii, 1.
[13]Gorham and Myers: Arch. Int. Med., 1917, xx, 599.
[14]Bock and Benedict: Jour. Biol. Chem., 1918, xxxv, 227.
[15]Myers: Jour. Lab. and Clin. Med., 1916, i, 178.

A THIRD MODEL ILLUSTRATING SOME PHASES OF KIDNEY SECRETION*

By Martin H. Fischer, M.D., and George D. McLaughlin, Cincinnati, Ohio

I. INTRODUCTION

IT has been emphasized upon previous occasions[1] that it is necessary in discussing secretion to differentiate between (1) secretion of water and (2) secretion of the substances "dissolved" in the water. Secretions, in other words, are not formed "as such," but represent the result of those interacting circumstances which make primarily for the secretion of water and secondarily for what has been called a leaching out of available substances to be dissolved in the water. While secretions are usually thought of as materials strangely different from gland substance or from blood, this difference is more apparent than real. Secretions do not in general differ from their sources except in *quantitative* composition. A secreting cell may, of course, elaborate a new chemical entity, and in this way contribute to a secretion something not found in the blood, but, except for such, it is only a matter of the same or more or less of urea or salt or acid in the urine than in the kidney, or, to trace it all back to the first source, in the blood itself. Why such quantitative differences come to pass requires no recourse to vague "vitalistic" or "physiologic" concepts—mere differences in the distribution of dissolved substances (as determined by differences in solubility, in adsorption or in chemical composition) between three phases of different chemical or physical make-up (in other words, the secretion, the secreting parenchyma, and the blood) are sufficient to explain even the most marked of the existent quantitative differences.[2]

We purpose returning in these paragraphs to that half of the secretory process which has to do with the mechanism by which a glandular parenchyma separates *water* from the blood and thus fathers a secretion.

If we ignore the entirely purposeless efforts to explain such separation on a "physiologic" basis—which amount in essence to nothing more than the statement that a cell secretes because it secretes and that it fails to secrete because it can not—then the attempts to account for what is observed may be divided into two classes. The first of these is the filtration theory originally put forth by Bowman (as mere speculation based upon histologic studies) and later by Carl Ludwig and his followers upon an experimental basis. The second is properly not a theory at all, but really only a critique of the Bowman-Ludwig concept by R. Heidenhain and his successors, who, on the basis of various physiologic observations (in which the questions of the secretion of water and the secretion of various dissolved substances are hopelessly confused) negate the adequacy of the filtration hypothesis. A good illustration of the modern situation is furnished by the work of T. G. Brodie and his coworkers[3] who, from their experiments on urinary secretion, came to the conclusion that the secretion of water by such an organ as the kidney is paralleled by a consumption of oxygen. At times there was also an in-

*From the Eichberg Laboratory of Physiology in the University of Cincinnati, Cincinnati, Ohio.

creased carbon dioxide production. In plainer terms, such a parallelism means that in secreting water the kidney does work, that this work calls for a consumption of energy, and that the greater the amount of work thus done, the greater the consumption of energy. We have ourselves supported such a view.[4] The work of other authors is, however, opposed to such a conclusion, notably that of J. Barcroft and H. Straub[5] who noted an even twenty-fold increase in water output by the kidney, under the influence of injections of various salt solutions, with *no* appreciable increase in oxygen consumption. As must be apparent, the two observations are mutually exclusive for were they both correct, it would compel the conclusion that to secrete water the kidney must sometimes do work and sometimes not. Is there a less inconsistent way out?

In our opinion there is, provided we will stop confusing the two elements of the secretion of water and the secretion of dissolved substances, and bear in mind the conditions experimentally found necessary for every increase or decrease in the secretion of these two materials and the significance of such conditions in the body of the living animal from a colloid-chemical point of view.

Our previous studies have shown that the conditions making for the *absorption* of water by the colloid-chemical mass which we term a living animal, are the opposite of those which make for the secretion of this same substance. Thus water is absorbed only as the capacity of the body colloids for holding water is increased by any of various circumstances, while a separation or secretion of water follows as these conditions are reversed. Water is therefore best absorbed in those portions of the alimentary tract (the large intestine) which are bathed by the most venous blood (that richest in carbonic or other acids) while no such absorption occurs in the stomach during the "periods of digestion" when this viscus is supplied with highly arterialized blood. A *secretion* of water, after its absorption into the venous blood, becomes possible as soon as the acid content of this venous blood (carbonic acid under normal circumstances or other acids in states of disease) is diminished on passing through the lungs. At this point water becomes "free" and may be lost through any secretory organ. A first place of loss is in the lung itself, while the other places of loss of general importance are represented by the kidney and the sweat glands of the skin. To make possible a secretion of water, the following conditions must, therefore, be satisfied: (1) "free" water must be brought to the secreting parenchyma, (2) the blood carrying such free water must contain a sufficiently low concentration of various acids (and a sufficiently high concentration of oxygen) and (3) if the secretion of water is to cost the kidney or other secreting gland no work the secreting parenchyma must be "permeable" to the free water and not to the combined.

Upon previous occasions we have shown how the experimental data of other authors, as well as our own all support the first two of these conclusions. A water-starved animal is incapable of any secretion; and the injection of any amount of water in combined form (as whole blood, blood plasma or any proper hydrophilic colloid)* will not increase any secretion by a drop. Secretion in-

*This constitutes the scientific basis for the injection of proper colloid solutions to raise and maintain blood pressure in shock. It seems to be largely forgotten by modern writers on this subject that the principles underlying the use of such solutions and the relative merits of blood, blood serum, gelatin and acacia mixtures were worked out in this laboratory over a decade ago. See Fischer, Martin H.: Oedema, New York, 1910, p. 186; Kolloidchem. Beihefte, 1911, ii, 324; Hogan, J. J., and Fischer, Martin H.: Kolloidchem. Beihefte, 1912, iii, 411; Fischer, Martin H.: Oedema and Nephritis, New York, 1915, ed. 2, p. 345; Hogan, J. J.: Jour. Am Med. Assn, 1915, lxiv, 721.

creases and is proportional to the amount of "free" water furnished the secreting organ (in other words, the amount available above that necessary for saturation of the colloids). When secretion-promoting substances like saline diuretics or materials of the type of caffeine and digitalis are used, the mechanism still remains in essence the same. In the first instance, the administered salts dehydrate the body colloids generally, and thus make available free water for secretion, while, in the second instance, they increase cardiac and respiratory efficiency, and by thus increasing the circulation through the tissues, decrease their content of carbonic and other acids, thus again making for the appearance of the free water necessary for secretion.

These ideas may be applied to such experiments as those of Brodie and his followers, and Barcroft and Straub. While insisting upon the general parallelism between amount of oxygen consumed and water secreted a study of the individual protocols and foot-notes of the first named of these authors indicates that he himself failed to discover such parallelism whenever a diuretic salt was administered; and this is the rule in all of Barcroft and Straub's findings. We would, in our own terms say that these facts compel the conclusion that the secretion of "free" water does *not* cost a kidney or other secreting organ any work. This conclusion is the logical one demanded by the colloid-chemical theories of absorption and secretion for which we have so long stood, but because such apparently finite experiments and deductions as those of Brodie stood against us we, too, insisted that water secretion represented an "active" process,[6] and thus failed to find in filtration and the "microcapillary" structure of the colloids (as illustrated in our first urinary secretion model[7]) an explanation of *all* the phenomena observed in a living animal. Therefore, if the secretion of water is essentially a filtration process, simple hydrated colloids of proper composition ought to behave like kidney parenchyma. The experiments described in this paper show that they do.

Since the colloid-chemical behavior of various hydrated soaps seems to be identical in every way thus far studied with the behavior of that hydrated protein mass which we call protoplasm (more specifically, for our purposes, with the parenchyma or hydrated colloid membrane which lies between any secretion like urine and its source, the blood) we decided to study the filtration properties of such hydrated soap systems in order to discover whether they showed any analogy to what is observed in biologic secretion.

II. EXPERIMENTS

We chose for particular study the system, hydrated sodium stearate. For filtration purposes this was cast into cylindrical cups measuring in the moist condition 7.2 cm. transversely by 5.2 cm. high with walls 1 cm. thick. The cups were made by supporting one calibrated beaker (of 120 c.c. capacity) within a second (of 350 c.c. capacity) and filling the intervening space with a hot sodium stearate solution. After the material had "set" by being cooled to room temperature, the soap model was removed from the mold and set up for experimental purposes as indicated in Fig. 1 except that the whole was covered so as to prevent undue evaporation. In the experiments to be described, the filtration properties of the cups were then tested by filling them with 80 c.c. of the solution to be filtered. The filtration pressure (the hydrostatic pressure) represented by this volume of fluid is 5 cm.

Water filters through such a soap cup in a fashion remarkably analogous to that observed in the various secreting organs of the body.

1. We tested out first the effects of the concentration of the hydrated sodium stearate system upon the filtration of water through it. The amounts of water which will pass through a sodium stearate cup of different concentrations under the circumstances described for these experiments are indicated in Table I.

Fig. 1.

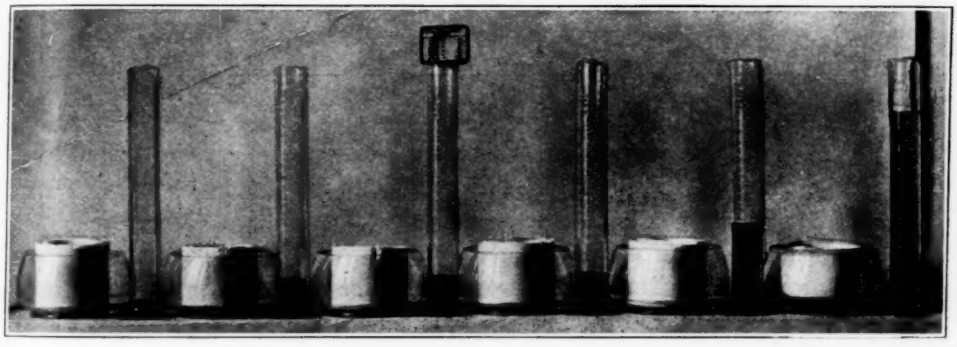


Fig. 2.

TABLE I

AMOUNT OF WATER WHICH FILTERS THROUGH SODIUM STEARATE CUPS OF DIFFERENT CONCENTRATIONS

HOURS ALLOWED FOR FILTRATION	CONCENTRATION OF SODIUM STEARATE CUP					
	1/2 m	2/5 m	3/10 m	2/10 m	1/10 m	1/20 m
2:45	0	0	0	0	3 c.c.	27 c.c.
4	1 c.c.	2 c.c.	1 c.c.	1 c.c.	6 "	34 "
5	1 "	3 "	3 "	2 "	8 "	42 "
6	2 "	3 "	4 "	2 "	10 "	46 "*
8	3 "	5 "	5 "	4 "	14 "	62 "
24:15	3 "	10 "	11 "	12 "	36 "	96 "
NEUTRALIZATION VALUE IN C.C. n/10 H_2SO_4 OF WHOLE FILTRATE	.1 "	.5 "	.6 "	6 "	1.2 "	1.4 "
NEUTRALIZATION VALUE PER C.C. OF FILTRATE	.033	.050	.054	.050	.033	.014

*25 c.c. more H_2O added to this cup.

These experiments show that the ease with which water filters through a hydrated sodium stearate decreases with every increase in the concentration of the colloid membrane. The filtration capacities of the different soap cups are so strikingly different that they are readily apparent to the naked eye as shown in Fig. 2, which is a photograph of this experiment at its end.

2. It was our next purpose to demonstrate the gross differences existent between the rate at which "free" water will filter through such a colloid cup and combined water as represented by a liquid colloid which in its physical constitution may be compared with blood. To this end we compared the filtration of water with that of a molar "solution" of sodium oleate. Since in the previous series of experiments 1/10 m sodium stearate cups had yielded a good average filtration rate we chose these for this experiment and the subsequent ones.

TABLE II

AMOUNT OF WATER WHICH FILTERS THROUGH 1/10 MOLAR SODIUM STEARATE CUPS WHEN "FREE" OR "COMBINED" WITH A HYDRATABLE COLLOID

HOURS ALLOWED FOR FILTRATION	COMPOSITION OF LIQUID BEING FILTERED	
	H_2O	1/m sodium oleate
:30	2 c.c.	0 c.c.
1:15	6 "	0 "
2:15	9 "	0 "
3:15	15 "	0 "
4:15	19 "	0 "
5:15	23 "	0 "
20	30 "	0 "

This experiment shows that water when combined with a hydrophilic colloid fails to come through a hydrated soap just as urine fails to come from blood when this contains no "free" water. That it is nothing specific about the sodium oleate which makes the filtration of water impossible but merely the fact that the water is bound to the sodium oleate is proved by the fact that mere dilution of the sodium oleate, sufficient to guarantee the presence of free water, at once allows some liquid to filter through. Its amount in no instance, however, equals that when pure water is used.

3. These colloid soap cups may be used to illustrate even the biological action of the various salines in increasing secretion. The saline diuretics owe their effects[8] to a dehydrating action upon the hydrophilic (protein) colloids of the body in general and upon those of the kidney in particular. By dehydrating the proteins of the body in general, they furnish "free" water for the kidney to secrete, while through such action upon the kidney specifically, they not only exhibit this function, but through it, obviously, the diameter of the capillaries must be increased which must be existent in the glandular parenchyma if mere filtration is presumed to be the mechanism by which free water is squeezed off from the blood.

The effects of the salines are different both as to their concentration and to their kind. Table III illustrates upon colloid soap how the "diuretic" effect of any salt is increased with every increase in its concentration.

The results of this experiment at the end of 7 hours are shown in Fig. 3.

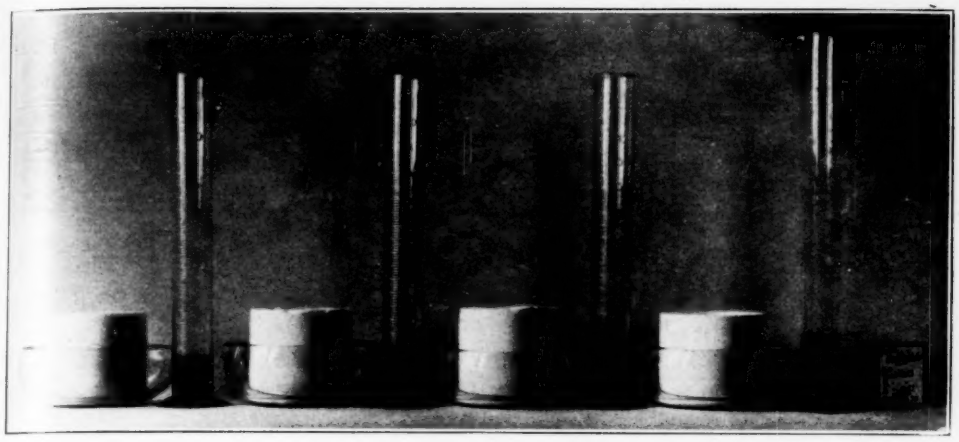


Fig. 3.

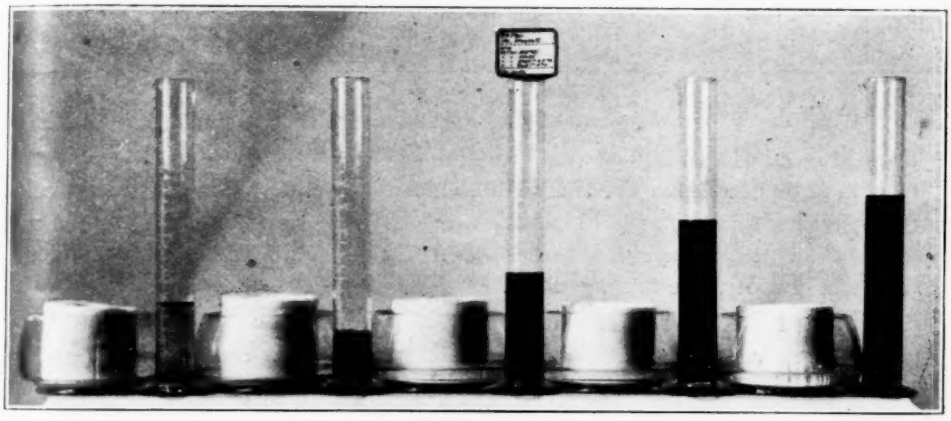


Fig. 4.

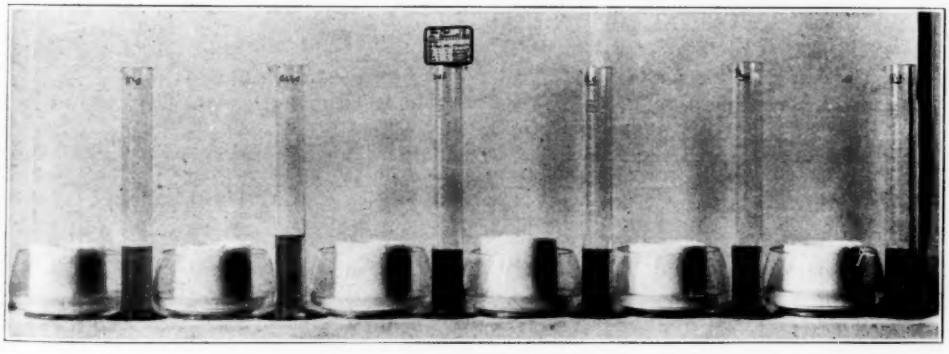


Fig. 5.

4. Table IV illustrates the difference in effects when equally concentrated (equimolar) solutions of salts possessed of a common acid but different basic radicals are filtered through a series of cups.

TABLE III

AMOUNT OF WATER WHICH FILTERS THROUGH 1/10 M SODIUM STEARATE CUPS FROM PURE WATER AND SOLUTIONS OF SODIUM CHLORIDE OF DIFFERENT CONCENTRATIONS

HOURS ALLOWED FOR FILTRATION	COMPOSITION OF LIQUID BEING FILTERED			
	H_2O	1/8 m NaCl	1/4 m NaCl	1/1 m NaCl
4	10 c.c.	13 c.c.	15 c.c.	20 c.c.
7	16 "	19 "	22 "	27 "

TABLE IV

AMOUNT OF WATER WHICH FILTERS THROUGH 1/10 M SODIUM STEARATE CUPS FROM EQUIMOLAR SOLUTIONS OF DIFFERENT SALTS

HOURS ALLOWED FOR FILTRATION	COMPOSITION OF LIQUID BEING FILTERED				
	H_2O	1/8 m NH_4Cl	1/8 m NaCl	1/8 m $MgCl_2$	1/8 m $CaCl_2$
:30	1 c.c.	3 c.c.	3 c.c.	3 c.c.	4 c.c.
1:15	2 "	6 "	8 "	10 "	10 "
2:15	6 "	9 "	14 "	20 "	23 "
3:15	9 "	12 "	20 "	29 "	35 "
4:30	15 "	14 "	27 "	40 "	50 "
5:30	19 "	16 "	33 "	52 "	64 "
6:30	23 "	17 "	38 "	58 "	70 "
7:50	30 "	18 "	42 "	62 "	72 "

The results of this experiment at its end are shown in the photograph of Fig. 4.

It is to be noted that the ammonium chloride leads to a *decrease* in secretion as compared with the effects of pure water. On the other hand, magnesium and calcium chloride are more powerful "diuretics" than sodium chloride. We return to a discussion of these findings later.

We tried next the effects upon filtration of equally concentrated (*either equinormal or equimolar*) solutions of salts containing a common basic radical with different acid radicals. Table V gives the results when equimolar solutions are used, while Table VI and Fig. 5 give the findings when equinormal solutions are employed. It will be observed that within the limits of experimental error there is *no change* in either case in amount of water secreted as compared with

TABLE V

AMOUNT OF WATER WHICH FILTERS THROUGH 1/10 M SODIUM STEARATE CUPS FROM EQUIMOLAR SOLUTIONS OF DIFFERENT SALTS

HOURS ALLOWED FOR FILTRATION	COMPOSITION OF LIQUID BEING FILTERED					
	H_2O	1/8 m NaCl	1/8 m Na_2SO_4	1/8 m Na acetate	1/8 m Na_2HPO_4	1/8 Na citrate
1	1 c.c.	1 c.c.	2 c.c.	1 c.c.	1 c.c.	1 c.c.
2:30	3 "	5 "	4 "	2 "	3 "	3 "
3:30	5 "	7 "	6 "	4 "	4 "	5 "
5:30	8 "	12 "	10 "	7 "	7 "	10 "
6:30	11 "	14 "	12 "	9 "	8 "	14 "
7:30	12 (?)	16 "	13 "	11 "	9 "	17 "
24:10	37 (?)	37 "	35 "	31 "	30 "	cup broken
NEUTRALIZATION VALUE IN C.C. n/10 H_2SO_4 FOR WHOLE FILTRATE	1.4 c.c.	.7 c.c.	.7 c.c.	.6 c.c.	.8 c.c.	

TABLE VI

AMOUNT OF WATER WHICH FILTERS THROUGH 1/10 M SODIUM STEARATE CUPS FROM EQUINORMAL SOLUTION OF DIFFERENT SALTS

HOURS ALLOWED FOR FILTRATION	COMPOSITION OF LIQUID BEING FILTERED					
	H_2O	1/8 m NaCl	1/16 m Na_2SO_4	1/8 m Na acetate	1/24 m Na_2HPO_4	1/24 m Na citrate
1	1 c.c.	2 c.c.	2 c.c.	1 c.c.	1 c.c.	1 c.c.
2	2 "	4 "	3 "	1 "	2 "	2 "
3	4 "	6 "	4 "	2 "	2 "	2 "
4	5 "	7 "	6 "	4 "	4 "	4 "
5	6 "	9 "	7 "	5 "	5 "	5 "
6	8 "	11 "	8 "	7 "	7 "	7 "
7	9 "	13 "	9 "	8 "	7 "	8 "
22	30 "	32 "	27 "	28 "	24 "	28 "
NEUTRALIZATION VALUE IN C.C. n/10 H_2SO_4 FOR WHOLE FILTRATE	1.3 c.c.	.7 c.c.	.8 c.c	.7 c.c.	.8 c.c.	.5 c.c.

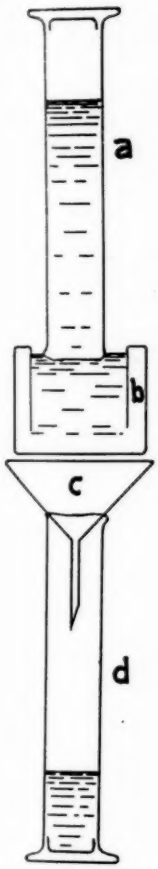


Fig. 6.

the effects of sodium chloride. In other words, the greater diuretic action of the acetates, citrates, phosphates and sulphates observed in living animals as compared with the diuretic action of equally concentrated chlorides, bromides, or iodides does *not* appear in these filtration experiments with soaps. To the discussion of this finding we also return below.

6. There exists a variable in all the described experiments which may be made a constant when it is so desired. In the simple arrangement described above, the filtration pressure falls as the amount of liquid which passes through the cup increases. Since, besides, the filtrate is not removed as formed, more and more accumulates about the cup and thus reduces the surface available for filtration. Both objections may be overcome by utilizing the filtration arrangement with constant level shown in Fig. 6.

In Fig. 6 *b* represents in section a sodium stearate cup supported upon a coarse galvanized iron screen held in a ring stand. In proper position above the cup is supported the inverted 100 c.c. graduate *a*. At the beginning of the experiment the cup *b* and the graduate *a* are filled with the liquid to be filtered, the filled graduate being inverted and placed in position as indicated in the figure. The filtration pressure is obviously determined by the height of the liquid standing in *b*. As liquid filters through the cup and its level falls in *b*, air enters the graduate, allowing the liquid column in *a* to fall sufficiently to restore the old level in *b*. In this way the filtration pressure available in *b* is kept constant. Any liquid which filters through *b* is caught by the funnel *c* and collected in the graduate *d* from which direct readings as to quantity of filtrate may be made.

III. DISCUSSION

1. The model described above may be used also to demonstrate various facts regarding *the secretion of dissolved substances.* A first point covers the matter

of how a secretion more alkaline may be derived from a less alkaline or even neutral source (parenchyma or blood). If phenolphthalein is added to the solution being filtered, is applied to the soap of the cup, and is added to the secretion escaping from the soap cup, in such an experimental series as indicated in Table III (where water and sodium chloride solutions of different concentrations are being filtered) the following facts may be observed. The original salt solutions leave phenolphthalein uncolored; so also do the sodium stearate cups; in the case of the filtrates, however, that from pure water turns the phenolphthalein brilliantly red. The same is true, though in decreasing intensity, as we examine the "secretions" from the cups containing increasingly strong sodium chloride solutions. The filtrate from the sodium chloride of highest concentration may leave the phenolphthalein practically uncolored.

Expressed in ordinary terms, alkaline secretions are here being derived from parenchymas or blood which are by themselves neutral or even acid. There is, however, nothing mysterious in the observed facts if a previous study on the composition of the lyophilic colloids and of the behavior of the colloid soaps toward indicators is kept in mind.[9] Distilled water and aqueous solutions of neutral salts are of course not expected to color phenolphthalein. The solid soap cups are essentially solutions of *water in sodium stearate* and such hydrated soap colloids also leave phenolphthalein uncolored. But upon diluting such a colloid, the soap dissolves in the water and such solutions of *soap in water do* color phenolphthalein. Hence the secretion which has passed *through* the solid sodium stearate is "alkaline" in reaction. This solubility of the soap in water decreases, however, as neutral salt is added to the water, hence less soap dissolves and hence less reaction to the phenolphthalein.

The biological significance of these findings (aside from the light which they throw upon the mechanism by which secretions more or less acid or alkaline may be obtained from allegedly more neutral sources) is of course great. In the terms of the pure physical chemists, the dilute soap solutions, as represented by the filtrates, are alkaline because, after hydrolysis of the dissolved soap, there is present an excess of hydroxyl ions. By the same reasoning the soap cups show no reaction to phenolphthalein because these are too "concentrated soap solutions." Even if we allow the correctness of such deductions—we are ourselves of the opinion that indicator methods are largely inapplicable here*—the necessary additional conclusions are still of small comfort to orthodox physiology. From a colloid point of view protoplasm behaves like the soap cup and blood and lymph like a concentrated liquid colloid of the type of sodium oleate. By the indicator methods these show no ions. Are the physical chemists who believe in the applicability of the laws of dilute solutions to living cells going to admit that solid protoplasm and blood and lymph under normal circumstances contain practically no ions, and that they have in consequence ascribed to the theories of dilute solutions, electrolytic dissociation, etc., an importance which they in no sense can exercise in normal living tissues? We believe, as a matter of fact, that normal *uninjured* protoplasm is, from a physical point of view, electrically neutral and that there are in it under normal circumstances as few or less electrically charged

*Because to our minds the system *soap dissolved in water* is something totally different from the system *water dissolved in soap*.

atoms and groups of atoms as in pure water or alcohol or ether or a dry crystal holding water of crystallization. Protoplasm is, in other words, a "solution" of water in hydrophilic colloid and this is a system to which the physico-chemical laws covering the dilute solutions may not be applied without the greatest reserve. Such laws may only be applied to the solutions of protoplasm in water, as to the more watery secretions from the body like urine, saliva or sweat.

2. If, instead of testing the "secretions" in the above experiments for alkalinity, we analyze them for content of fatty acid (in other words, for amount of soap dissolved in the secretion), it is found that most soap is dissolved when plain water is filtered through the cup and less and less as salt solutions of increasing concentration are employed. This too has its biological parallel. Distilled water can not, for example, be furnished secreting cells without damaging them, for the cells tend to dissolve in such distilled water. Thus the excessive consumption of distilled water makes for the appearance of albumin in the urine. To keep a colloid parenchyma (be it soap or protoplasm) from thus going into solution, salt must be added to the distilled water. This is one of the reasons why all cells are less damaged by a so-called physiologic salt solution than by plain water. In fact in the case of a kidney previously damaged by distilled water (or worse still by some powerful "solution"-producing agent like an acid) the progressive solution of the kidney in the urine (the albuminuria) may be cut down by perfusion of the organ with a proper salt solution.

3. We wish finally to return to the fact that if the rate at which distilled water filters through a soap cup is taken as the standard, the rate of such secretion from an ammonium chloride solution is less, while that from other salt solutions like sodium, magnesium or calcium chloride at an equivalent concentration is greater and in the order named. This, too, has its analogue in what may be observed in animals, and the explanation of what occurs in animals may be deduced, we think, from the action of these salts upon soap. Generally speaking all salts tend to dehydrate soaps and this in increasing amounts with increasing concentration. When sodium chloride is used there is therefore a progressive increase in dehydration of the hydrated sodium stearate constituting the soap cup and with the secondary enlargement of the capillary pores consequent upon this, filtration is made proportionately easier. When, however, a salt capable of entering into double decomposition with the sodium stearate is used, there is added to this first effect a secondary one incident to the production of a new soap. The hydration capacities of soaps of the commoner fatty acids with different bases runs in about the following order:

NH_4, K, Na, Mg, Ca.

(The solubility of these soaps *in* water also runs in this order). It will now become clear, when equally concentrated solutions of these different salts are filtered through sodium stearate, why ammonium and potassium salts lead to a lesser secretion of water than sodium, while magnesium and calcium salts lead to a greater one, and in the order named. After double decomposition and at least partial formation of the corresponding soaps, the original capillarity of the hydrated sodium stearate will be changed, being decreased in the first named, left largely unchanged in the middle member, and increased in the last named.

It was emphasized above that while the "diuretic" action of equally concentrated but different salts differs when their basic radicals are different, such distinctions are largely missing when salts with a common base but different acid radicals are employed. In other words, these soap models do not show the greater diuretic action of the citrates, tartrates, phosphates and sulphates over chlorides, for example, which do animal kidneys or living cells in general. We expected this result as the necessary corollary of certain chemical differences between fatty acids and amino acids and the possibilities possessed by the latter of forming longer series of different salts.

The action of different bases upon a fatty acid may be compared with the action of the same bases upon the polymerized amino acid which we call protein. "Soaps" are formed in both instances with their varying hydration capacities. It is our belief, in other words, that if we write the formula for any fatty acid as:

$$x - COOH$$

that the colloid-chemical properties of the different soaps are dependent upon the substitution for the H in the above formula of the various metal radicals. If now we write the formula for an amino acid as:

$$\begin{array}{l} x - COOH \\ | \\ NH_2 \end{array}$$

the effects of the metal radicals are again the same, and the colloid-chemical behavior of the pure proteins which are thus formed is again of the same kind as in the case of the soaps. Instead of soaps of fatty acids we get "soaps" of proteinic acid, the solvation and solution characteristics of both of which are largely the same. The fatty acids do not, however, possess the power of uniting with acid as do the amino acids. In the latter instance acids may unite with the NH_2 groups. In this fashion there can then be produced the chlorides, bromides, sulphates, phosphates, etc., of the polymerized amino acids each again possessed of its own solubility in water and solvent power for water. Since the cells of the living body are the hydrated salts of polymerized amino acids, the various salts may affect them "diuretically" not only through their basic, but also through their acid radicals.

IV. SUMMARY

The question is raised of the mechanism by which *water* is secreted by such a secreting parenchyma as a kidney. The experimental evidence is reviewed which indicates that only "free" water can be separated from the blood and that the separation of such water costs the kidney no work. This supports the conclusion that such separation is a mere filtration process, and since the secreting parenchyma of such an organ as the kidney is a hydrated colloid which has properties closely akin to a solid hydrated soap, the filtration properties of such a soap (sodium stearate) are studied to see whether any analogy exists between its behavior and what may be observed biologically.

1. Hydrated sodium stearate allows water to pass through it under slight hydrostatic pressure, the ease of such passage being increased as the concentration of the hydrated colloid is lowered.

2. While "free" water passes readily through such a hydrated colloid, water tied to a hydratable colloid (liquid sodium oleate) can not.

3. Salt solutions lead to a greater filtration of water than plain water and this (a) according to their concentration and (b) their kind, generally speaking. The higher the concentration of any salt, the greater the filtration of water. On the other hand, at given concentration, salts of ammonium or potassium produce less filtration than salts of sodium, and these less than those of magnesium or calcium. The findings on soaps are here identical with the behavior of these same salts upon the living organism. When, however, the effects of equally concentrated salts with the same basic radical but different acid radicals are compared, *no* such diuretic differences appear as occur in living animals.

4. The theory of the action of these effects is discussed, it being pointed out that because of the existent differences in chemical composition of fatty acids and of the polymerized amino acids known as protein it is possible in the former to produce only one series of salts as different bases are introduced into the fatty acid. In the case of the proteins a similar series may be produced, but because of the existence in the latter of NH_2 groups, a second series may be produced through the linking of acid with these groups. Colloid-chemical and physiologic behavior are then an expression of the solvation and solubility properties of the different compounds thus formed.

5. The dangers of applying without due reserve indicator methods and the laws of dilute solutions of electrolytic dissociation, etc., to the normal cells and fluids of the body but not to their secretions is reemphasized.

REFERENCES

[1]Fischer, Martin H.: Oedema, 1910, New York, pp. 180, 200; Oedema and Nephritis, 1915, New York, pp. 286, 324.
[2]Fischer, Martin H.: Nephritis, 1912, New York, p. 113; Oedema and Nephritis, 1915, New York, ed. 2, p. 510; Jour. Lab. and Clin. Med., 1920, v, p. 207.
[3]Barcroft, J., and Brodie, T. G.: Jour. Physiol., 1904, xxxii, 18; ibid., 1905, xxxiii, 52.
Brodie, T. G., and Cullis, W. C.: Ibid., 1906, xxxiv, 224.
Brodie, T. G.: Harvey Lectures, Philadelphia, 1909.
Cushny, A. R.: Secretion of Urine, 1917, London, pp. 34, 35.
[4]Fischer, Martin H.: Oedema and Nephritis, 1915, New York, ed. 2, p. 507.
[5]Barcroft, J., and Straub, H.: Jour. Physiol., 1911, xli, 145.
[6]Fischer, Martin H.: Oedema and Nephritis, 1915, New York, ed. 2, p. 314.
[7]Fischer, Martin H.: Ibid., p. 283.
[8]Fischer, Martin H.: Oedema, 1910, New York, p. 134; Oedema and Nephritis, 1915, New York, ed. 2, p. 295.
[9]Fischer, Martin H.: Science, 1918, xlviii, 143; ibid., 1919, xlix, 515; Chemical Engineer, 1919, xxvii, 184; ibid., 1919, xxvii, 271.

THE EPIDEMIOLOGY OF INFLUENZA-PNEUMONIA*

By Colonel Charles Lynch, Medical Corps, U. S. A., and Lieut.-Colonel James G. Cumming, Medical Corps, U. S. A. Port of Embarkation, Newport News, Va.

IT is noteworthy that as a result of the influenza epidemic the pneumonia death rate in civil life was 4.7 per 1000 population, while in the army this rate was 14.4 per 1000—a ratio of 1-3 comparing the two groups; in other words, per 1000 population, there were three times as many deaths in the army as in civil life.

What is the reason for this difference in the mortalities of the two groups? Is it age group susceptibility, air space, adequacy of clothing, fatigue, habits, crowding, food, or insanitary messing and other indirect contact incident to army life?

It has been argued because of the extreme prevalence of influenza in the military service and supposedly among civilians in the same age group that this age group is particularly susceptible, in short, that this is the susceptible age group. In order to get some definite data on this question certain information concerning children in public institutions, was secured.[1] As a result of this investigation it was found that among these children, 49,140, the influenza rate was 412 per 1000. Among 703,006 adults in similar institutions the rate was 203 per 1000. It should be added that these 49,140 children constituted the population of 131 institutions. These figures include children in a large number of institutions scattered throughout the United States. Since we found the rate highest among children it is obvious that in the young, we have the susceptible age group. This might be expected for in children we have a larger percentage of nonimmunes, while the adult population as a whole is partially immune as a result of previous attacks.

It is a fact that during the early part of the epidemic the disease prevailed most extensively among male adults, but was not this due to exposure to infection incident to their mode of life? In the days of typhoid prevalence it was recognized that young male adults, owing to their greater exposure—they eating in public places and obtaining their water from many sources—developed this disease more frequently than any other group. We have an analogy to this in influenza. The young adult is more subject to exposure owing to his mode of life. Fundamentally, infection results only from the introduction of the specific organism. Exposure is the primary factor; the secondary being the susceptibility of the individual, and this depends largely on the immunity conferred by a previous attack.

On this hypothesis we would expect the highest influenza rate in children, and the lowest among the aged. An investigation of the rates in public institutions confirms this theory.

*Read before the Medical Section of the American Life Convention, Chattanooga, Tenn., March 26, 1919.

Authority to publish granted by Surgeon General's Office, Washington, D. C.

As a matter of fact all nonimmune individuals are susceptible whatever their age group, and will develop the disease upon the introduction of the infective agent, so that fundamentally the object to be sought is prevention of infection.

Epidemiologic studies show very definitely that air space, within moderate limits, adequacy of clothing, fatigue, habits, the kind and adequacy of food and water, play little or no part in the spread of the so-called respiratory diseases.

ARMY INFLUENZA

This leaves only insanitary messing and other indirect contact, and our recent laboratory studies and field investigations of troops show that in them the respiratory diseases are spread by indirect rather than by direct contact. Droplet infection in camps, in civil life, and in hospitals is believed to play but a minor role in the spread of sputum-borne diseases.

Indirect transmission of infection takes place by two routes of travel. But a distinction must be made between transmission in civil and that in army life. In the army the insanitary methods of washing mess gear were chiefly responsible for the high rates of sputum-borne infections. Unclean hands were then an important factor in the army.[2]

Each soldier washed his own mess gear in the same water, used by other soldiers in the same company. The companies were large—250 men. The water was inadequate in amount and with the washing of each successive mess gear it became more highly contaminated with mouth organisms from the whole company. These organisms were introduced from the mouth to the mess-kit wash water by two routes, the mess gear and the hands. The wash water was warm, sometimes hot, but the infective agents transferred to it were not killed, as it was not hot enough to do this. There was a cumulative action; the pollution of the water increased with the successive washing of mess gears, pollution by mouth and hand organisms. The washed mess gear and hands were thus contaminated by organisms from the mouths of many men. The organisms, newly acquired by the water, travel from the wash water to the mouth through two routes, through the eating utensils and the hands. From the frequency with which the hand visits the mouth it is more than probable that this is the major route.

The mess kit wash water is the focus; its use gives a common contact among troops, and this mess kit wash water is the most intimate and frequent point of indirect contact transmission for a group of mouths. Close observation of the messing of troops leads one to the above hypothesis. But have we any proof that this is actually so, and if so will it be possible to institute preventive measures? As troops were messed by two different methods it was possible to collect evidence which proved this hypothesis satisfactorily. For aside from troops, which eat from mess-kits, which they themselves wash, many organizations eat from tableware which is washed by the kitchen force. Under the latter conditions the contamination of the hands of each soldier by the common wash water, is eliminated. His hands are not exposed to the pollution by organisms common to dish water. He should then be more likely to escape infection. An investigation of the incidence of influenza among 66,000 troops, 31,000 of whom ate from tableware, and 35,000 from mess kits, proves that those who ate from tableware, which is washed by a limited force, were far less liable to infection.[3] The rate of

infection in this group was 51 per 1000, while in that group which ate from mess kits, washed by the men themselves, the rate was 252 per 1000. The ratio in the two groups is 1 to 5, or per 1000 troops in the two groups, 84 per cent of the cases occurred among those whose hands were contaminated by the washing of their own eating utensils. It would seem that this is ample proof of our original hypothesis that the infection occurred by indirect contact through the use of tepid eating utensil wash water.

In order to institute corrective measures, which will prevent to a large extent the high mortality among troops, of foremost importance is the providing of boiling water for washing eating utensils. There is then no cumulative contamination of the water, infective organisms are killed, the eating utensils are more thoroughly cleansed, they are disinfected, and of far greater importance is the fact that hand contamination is prevented because of the use of boiling water.

CIVIL AND INSTITUTIONAL INFLUENZA AS AFFECTED BY THE METHOD OF WASHING DISHES

What then we consider proved up to the present stage in our paper is that the excess of influenza in troops, and excess mortality, were due to their promiscuity in messing, or in other words, to increased indirect contact from this cause.

Interesting and important as this is so far as the army is concerned, does it not possess a wider significance? The results of further study demonstrate that this is actually the case.

In order to extend the study of the theory of indirect contact transmission of influenza to the civil population and to apply it directly to the question of feeding, investigations were made in Chicago and Washington.

Machine Washed

	Persons	*Cases*	
Chicago Employees 12 Hotels & Restaurants	1350	13	
Chicago Employees 4 Department Stores	13500	181	
Washington Employees 2 Department Stores	2386	155	
	17236	349	20 per 1000.

Hand Washed

	Persons	*Cases*	
Chicago Employees 3 Restaurants	25	7	
Chicago Employees 1 Department Store	3100	259	
Washington Employees 2 Department Stores	1050	163	
	4175	429	103 per 1000.
Total	21411		

These 21,411 business men and women were employed in fifteen hotels and restaurants, and in nine department stores, where all or a part of the meals were provided.

These employees were divided according to whether they ate from machine-washed or hand-washed dishes. Among 17,236 who ate from machine-washed dishes there occurred 349 cases of influenza, while among 4175 who ate from hand-washed dishes were 429 cases. There was a rate of only 20 per 1000 in the former while in that group in which the dishes were not treated with boiling water. the rate was 103 per 1000. It appears that the chances of infection be-

tween the two groups are as one is to five, or exposure to infection among those eating from hand-washed dishes is five times greater than in that group which ate from the disinfected dishes.

Is not this a suggestion for sanitary dish washing by immersion in boiling water in the home, as well as in public eating places, and does it not give us a new conception of what we mean by crowd disease?

It was maintained that on the Panama Canal pneumonia was brought under control by segregating in huts the workmen with their families. The crowding in barracks was thereby eliminated, but it is to be pointed out that at the same time the common mess as well was abolished. In the light of our recent epidemiologic knowledge it is believed that the beneficial results were mainly from the abolition of the *common mess* rather than from the change in lodging arrangements.

The results in the two civilian groups studied dictated an extended survey of civilian fixed groups. Inasmuch as state and federal institutions have such fixed groups and would be able to furnish accurate data as to the recent influenza epidemic, questionnaires covering the information desired, were sent to such institutions; 593 replies were received.

Many of these replies had to be discarded because they were incomplete or because no cases of influenza occurred in the institutions. Accurate data covering 252,186 individuals was secured, however.

In the group eating from machine-washed dishes the rate was 108 per 1000. while in the group eating from hand-washed dishes the rate was 324 per 1000. This gives a ratio of 1 to 3 between the two groups, or 75 per cent of the cases occurring in that group which ate from dishes not disinfected with boiling water, in other words, hand-washed dishes.

Here we have fixed groups living under the same institutional conditions, Can there be any other explanation for this striking difference in the influenza rates, except the notable difference in methods of dish washing, disinfection in the one case and increased contamination in the other?

DURATION AND DAILY RATE OF INFECTION

It is interesting to note that in institutions where machine-washed dishes were used the average duration of the epidemic was 5.1 weeks, whereas in institutions where hand-washed dishes were used this period was 4.5 weeks. The daily rate of infection then was three times greater in the hand-washed group.

In the machine-washed group the major route of transmission through eating utensils was eliminated, resulting in reducing the number of infections, but in prolonging the epidemic. In the other group, where both major and minor routes of transmission, eating utensils and inanimate objects, freely took part as transmitting agents, the duration of the epidemic was shortened and the daily rate of infection was three times as great.

These findings are wholly in keeping with our theory as to the relative importance of eating utensils and inanimate objects as conveyors of infection.

COMPARATIVE MORTALITIES IN THE TWO GROUPS

In the two groups we find that among those using machine-washed dishes the mortality from pneumonia per 1000 population was less than one-half the mor-

tality among those using hand-washed dishes. The difference in mortality is, in the ultimate analysis of any investigation, of prime importance. This especially holds true in our consideration of the influenza epidemic because influenza of itself but rarely kills. The recent pandemic might have come and gone without special comment and the fatalities would not have been more than a couple of thousand instead of 500,000 had the primary infection, influenza, been uncomplicated. Influenza, like measles and scarlet fever, is but rarely if ever the immediate cause of death, and reports of high death rates from influenza are to be accepted with caution.

The group of virus infections, influenza, measles, scarlet fever, are nonfatal primary infections. These prepare the soil for the highly fatal secondary invaders, the pneumococci and streptococci. It was then the pandemic of resultant pneumonia rather than that of the primary infection, influenza, which caused the heavy toll in deaths.

In normal times the body resistance is for the most part sufficiently high to ward off invasion by these secondary invaders which prevail in the throats of apparently normal individuals, producing a carrier state, a condition of potential danger to the carriers, as well as to others, because of the possibility of transmission.

These pneumonia-producing organisms lie in wait for the production by virus infections of favorable conditions for their invasion of lung tissue. The pneumonias are for the most part due to temporarily lowered resistance, a potential danger lingers in the throat of every carrier who harbors pneumococci or streptococci.

Was the high pneumonia death rate due wholly to the *absence* of a recent pandemic of influenza, or due entirely to what we may term recent increased promiscuity, increased communal intercourse, particularly community feeding? Of these two factors it is believed that community messing played the major role in this increased pneumonia mortality.

In former times community messing had its limitations. The working man carried his dinner bucket, now he goes to the restaurant. The clerk and the office man formerly returned home for lunch, now they patronize the cafeteria. Formerly when the farmer made a trip to town he took his lunch with him, but now he patronizes the restaurant. In the days of economy it was an event for even the first families of the community to gather at the hotel for a luxurious meal, and now that a premium is put on brawn even the laboring man considers a hotel party uneventful. But why should this increased patronage of common eating places influence the mortality from pneumonia?

Let us inquire further whether or not any advance has been made in our knowledge of the epidemiology of pneumonia as well as of the sputum-borne diseases? And let us not forget that upon this alone depends successful preventive measures. During the influenza epidemic the face mask was advocated and in many cities it was tried without beneficial results. Communities were quarantined against inter-city communication to no avail. All administrative effort on the part of the city, state, and federal health authorities was without result in limiting the spread of the contagion.

The quarantine of a city is impracticable. It can not be maintained over a sufficient length of time to be of value. There is only one example where quarantine has proved of practical value, and that is in state institutions where an absolute quarantine can be held in force for an indefinite time without jeopardizing commercial interests. There are a number of such institutions which escaped entirely by prolonged quarantine, and there are others in which a temporary quarantine resulted in the postponed admission of a convalescent or unrecognized case and spread of the contagion to practically all nonimmune inmates.

Influenza is highly contagious, but nonfatal, while pneumonia is moderately contagious, and, when the body resistance is temporarily lowered by a virus infection, highly fatal. In other words, the pandemic of influenza would be of no moment if unaccompanied by the fatal pneumonias.

Which of these infections is the more easily controlled, or are both controlled by the same preventive measures? Influenza virus is introduced and burns out quickly, while pneumonia organisms are continuously and insidiously spreading from carriers to nonimmunes where they prevail as a potential danger. The carrier state in question is not, as in the case of typhoid and immune diphtheria carriers, a danger only to others, but is a menace to the carrier as well. This distinction in types of carriers demands recognition by those interested in preventive medicine. In this connection let us study the comparative mortality per 1000 in New York City for 1890, 1891 and 1918.[4]

In comparing the pneumonia fatalities of New York during the influenza epidemics of 1890, 1891, with that of 1918, it is found that the mortality per 1000 population for the 1918 epidemic is twice as great as that of the former epidemics. Furthermore, the recent epidemic was of shorter duration, it was more explosive in character, indicating that our present day environment transmits with greater facility the infecting agent. With increased travel, increased eating in common eating places, increased congestion of urban districts, it may be concluded that the virus of influenza was transmitted with greater facility than ever before. With a large residual population of influenza nonimmunes, owing to the absence of a recent pandemic of the infection, and because of the increased facility with which the infective agent was transmitted, it may be further concluded that the recent pandemic has run its course, and that there will not be a second year recrudescence as in 1890 and 1891.

If one compares the mortality rates of cities in the registration area to the mortality rate for the entire population of the United States, it is found that the epidemic has spent itself, and assuming that the high mortality rates for the several years following 1890 and 1891 epidemics were due to an increased sporadicity of influenza-pneumonia, it may be concluded that the annual death rates following the recent epidemic will not be appreciably increased.

If the influenza virus is today transmitted with greater facility than in former times it is reasonable to assume that the organisms of secondary invasion are likewise more widespread than formerly; hence, we have this corresponding greater mortality from pneumococci and streptococci, secondary invasion resulting from a temporarily lowered resistance.

We have previously presented evidence showing that approximately 80 per cent of the influenza morbidity in the military service was due to insanitary

messing facilities. Corrective measures for these defects can be instituted and effectively carried out. Furthermore, in two large civilian groups it was shown that in civil life about 80 per cent of the cases of influenza occurred among those using hand-washed eating utensils. In addition we have shown that 75 per cent of the morbidity in institutional population occurred among those eating from hand-washed dishes.

It appears that in camps as well as in civil and institutional life the use of only warm water for washing eating utensils is the chief sanitary defect. In the camps the contaminated hand and its auxiliary, the eating utensil, are probably equally responsible for the dissemination of the contagion, whereas in civil and institutional population, the auxiliaries of the hand, eating utensils, are primarily responsible, while the contaminated hand aside from its auxiliary eating utensil, plays a less important role.

It would appear from epidemiologic data secured in our recent investigations that influenza, as well as scarlet fever, and the bacterial infections may occasionally be food borne.

Our investigations would indicate that transmission by droplet infection through the air route by direct contact is at the most only of minor importance as compared to transmission by indirect contact through the hand to mouth, or hand auxiliary to mouth route of travel. The fallacy of wearing the mask in civil communities and cubicling patients on a hospital ward, without disinfecting the hands might well be compared to a surgeon who would wear a mask and fail to wash his hands. Transmission of the sputum-borne diseases is indirect through the hand to mouth route of travel and in order to obtain favorable results in the control of any infection the major avenues of travel must first be blocked, and as the air route of transmission is of such minor importance, the value of the face mask fades into insignificance.

CORROBORATIVE LABORATORY RESEARCH

If the foregoing deductions, which are based almost wholly on field and hospital observations, are correct, it should be possible to verify them by laboratory research. In regard to our laboratory investigations covering both direct and indirect contact transmission of infective organisms, the following salient facts may be presented.

In a former report it was demonstrated that the epidemic of streptococcus pneumonia, which swept through the camps last winter, and which for the most part followed measles, was not a hospital infection, but that at least 94 per cent of the pneumonia cases following measles were streptococcus carriers upon entering the hospital.[5] From this finding it is a question whether the cubicle system, as carried out on the so-called respiratory disease wards, was of any value. It was reported by some investigators that throat swabs of measles cases on successive days showed from day to day an increase in the number of streptococcus hemolyticus cases, and on this was based the conclusion that the infection was spread in the hospital, hence the cubicle system of segregation.

Our former reports did not support these contentions, and our recent laboratory researches show the fallacy of those reports on which the hospital infection theory was based. Serial throat swabs from average throats of troops show

that the percentage of positive streptococcus carriers, while only 32 per cent on the first swabbing, may be as high as 82 per cent on the fourth swabbing, and this accounts for the apparent increase in positive cases on the hospital wards.

In all walks in civil life is there any more intimate and almost direct contact than at the public and private mess through the common spoon? The hand, contaminated by handling inanimate objects, and its auxiliary, the eating utensil, make up the busy avenues through which are spread the infective organisms of the sputum-borne diseases.

In infectious disease wards pathogenic organisms are invariably found in floor dust, while the examination of over 9000 cubic feet of air in these wards fails to reveal the presence of more than three hemolytic streptococci. By the spray and plate method it is found that even the finest spray has a rapid rate of fall. In three minutes 85 per cent of the organisms fall through a distance of two feet and in ten minutes the air is entirely free.

It then appears that the falling of even the finest spray expelled by an act of coughing may well be compared to an aeral barrage. Furthermore, we find that comparatively few organisms are expelled by coughing. By the sterile glove and cultural method it is found that this number rarely exceeds 1500 organisms.

From these findings it may be concluded that transmission by direct contact through the air route but rarely if ever takes place.

In contrast to this our recent research shows with what great facility organisms may be spread by indirect contact through inanimate objects by the hand to mouth route of travel. The number of organisms in the mouth runs into the billions; the number on the hand runs into the millions, and owing to the frequency of contact between the mouth and hands many hand organisms are of mouth origin. While only about 1500 organisms are expelled onto the floor by an act of coughing, a sterile glove wiped across the lips picks up about 2,000,000 organisms. Such organisms are readily transferred to inanimate objects which are handled by many people.

Hemolytic streptococci and pneumococci are isolated with great regularity from the hands of carriers or patients, from tableware, inanimate objects touched by these patients, and from floor dust, and diphtheria and tubercle bacilli have been isolated from the hands and eating utensils of patients.[6] These pathogenic organisms prevail so universally and in such number on the hands, on inanimate objects, especially on eating utensils, that the chances of transmission by indirect contact through the hand or hand auxiliary to the mouth, must outnumber the chance of spread through the air route by many hundred times. Furthermore, our epidemiologic data support this contention. We have secured most interesting and striking data in regard to the sanitation of washing dishes in both the home and public eating place. The average count of a large number of restaurant dishwater specimens is 4,000,000 bacteria per c.c. The temperature of this water is warm to the dish washers' hands, its average being 43° C. In restaurants where the hand method of washing is practiced the dishes are never scalded. The water is often so highly polluted that the dishes are more highly contaminated after they are washed than before the washing process. In the cycle which dishes make from table to kitchen and back again, the spoon or fork is often freer from

organisms just after being used by the restaurant patron than when taken from the restaurant's polluted dish water. In the average restaurant where dishes are hand washed only about 50 per cent of the organisms are removed by the washing process.

In children there is less than 1 per cent hemolytic streptococcus carriers. In those of the adolescent age, the percentage is slightly higher—1.2 per cent. In the civilian adults we find another increase to 5 per cent, while in the military forces there is that enormous percentage, by a single swab, of 42. In the Southern Department during the winter of 1917-1918, this organism was the most common of all secondary invaders, and in the winter months it caused a case mortality equal to that of former plagues. In our recently acquired knowledge of the facility with which transmission took place at the soldiers' mess, we have the explanation of epidemic pneumonia among troops.

To the cost of lives on the battle field there must be added those fatalities in the civil population which result from the inevitable post-bellum epidemic. After the Spanish American War there was an increased typhoid mortality; after this World War there will be an increased pneumonia death rate.

If pneumonia mortality has not heretofore been decreased in civil life by preventive measures, and if our report showing the increased influenza rate incident to the use of hand-washed dishes is of value as an index for the facility with which contagion may be spread at public eating places, it may be predicted that with the demobilization of approximately two million hemolytic streptococcus carriers, there will result in civil communities an equal number of new foci for the further spread of this potential danger. In addition to hemolytic streptococcus carriers, concerning which we have accurate data, it may be assumed that there is also a relatively high percentage of carriers of the other pneumonia-producing streptococci and pneumococci. This demobilization will of itself immediately and automatically increase the carrier state of hemolytic streptococci alone in the civil population to approximately 8 per cent. With these new foci, in conjunction with the increasing patronage of public eating places, we may predict an increasing number of carriers with the increasing mortality incident thereto in the civil population from year to year.

Preventive measures alone can stem this rising tide of increasing mortality. Among these the most important are the hygiene of the hand and the disinfection of dishes, but there are multitudes of other details.

In a large number of tests we have been able to isolate pathogenic organisms almost without exception from eating utensils used by carriers and patients. These results show the facility with which infective agents may be spread at the common mess and suggests the necessity of the disinfection by boiling water of all eating utensils. In public eating places this is best accomplished by the mechanical dish washer in which it is essential to use scalding water in order to get the cleansing effect. Our investigation of the dishes washed in this manner shows a minimum degree of contamination.

Much has been gained by the prohibition of the roller towel and the common drinking cup, but our work shows the necessity of extending this same idea to include common eating utensils. In fact we believe that today the greatest ad-

vance in preventive medicine can be accomplished by requiring in public eating places the disinfection of eating utensils. On the other hand, personal hygiene of the hand is a measure which the individual must regulate himself. Instruction in this, however, might well be undertaken in the habit-forming or school age.

There is nothing new in the theory of indirect contact, but the point is that as a transmitting avenue it has been almost wholly neglected, perhaps because of its simplicity. On the other hand the air-borne or droplet infection theory presented a complex problem capable of endless mysticism.

CONCLUSIONS

It is believed that the results of our epidemiologic studies and laboratory research show that indirect spread through the hand or hand auxiliary to the mouth is by far the most important and major route of contagion dissemination. Granting that this is true, preventive measures will not consist of periodic masking of the populace, but simply an intensification of the rules of personal hygiene, hand hygiene and especially the sanitation of eating utensils, and the protection of food. The most ordinary social requirements demand clean hands, clean eating utensils, and the protection of food supplies. But we must extend this to include the actual sterilization of eating utensils both in the army and in civilian life. In both, boiling water is essential for sanitary dish water. In civil life this should be provided in restaurants and the like by washing machines. In the army, if mess kits are utilized, the washing water must be boiling for two reasons; viz., to sterilize the mess gear and to prevent soldiers from putting their hands into it and so contaminating it or their hands as the case may be.

REFERENCES

[1]A detailed report to appear later.
[2]Lynch and Cumming: The Role of the Hand in the Distribution of Influenza Virus and the Secondary Invaders, Mil. Surgeon, December, 1918.
[3]Lynch and Cumming: The Distribution of Influenza Virus by Indirect Contact, Hands and Eating Utensils, Am. Jour. Pub. Health, January, 1919.
[4]Monthly Bull. Dept. of Health, New York, December, 1918.
[5]Cumming, Spruit and Aten: Streptococcus Pneumonia, Jour. Am. Med. Assn., Mar. 8, 1919.
[6]A detailed report of this research to appear later.

STUDIES ON PATHOGENIC ANAEROBES*

I. Biology of Bacillus Welchii

By Benjamin Jablons, Major, M.C., Army Medical School, Washington, D. C.

ENOUGH evidence has now been accumulated to prove that the B. welchii represents a large group of bacilli which are anaerobic and have a large number of group characteristics in common, but which differ from one another in what might be considered to be minor characteristics. Thus Simonds[1] has been able to differentiate them into four groups on the basis of their sugar fermentations and serologically there are at least two varieties which fail to agglutinate by means of specific sera. This organism has been incriminated in so many disease processes during the past two decades that it might be valuable to review their best known characteristics, and to describe in detail the pathologic lesions that this organism is capable of initiating, as a result of its toxic and other secretions.

This bacillus is identical with the following bacteria:

B. of Achalme (1891).[2]
B. aerogenes capsulatus, Welch and Nuttall (1892).[3]
B. phlegmonis emphysematosæ, Eug. Frænkel (1893).[4]
B. perfringens, described by Veillon and Zuber (1898).[5]
B. of Welch, Migulà (1900)[6] (1).

It is customary to call this organism in France the B. perfringens because it was the first binominal designation given to this microorganism, the name suggested by Welch being a trinominal one. In justice to Welch, we feel, however, that the name suggested by Migula in 1900 should be retained.

The causal role of the Bacillus welchii in gas infections had been demonstrated by Eugene Frænkel in 1893,[7] by Guillemot in 1898,[8] by Hitschmann and Lindenthal in 1899,[9] by Welch in 1900,[10] by Albrecht in 1902,[11] by Kamen in 1904,[12] etc. P. Simonds, in his monograph on the B. welchii published in 1915, collected 175 cases of gaseous gangrene or of gaseous phlegmon due to this microorganism, occurring before the war, with a mortality of about 45 per cent. This high figure indicates the importance of this organism in gaseous infections.

All authors agree that the bacillus of Welch plays an important role in the pathogeny of gas infections. Weinberg found this organism present more frequently than any other anaerobe in the series of gaseous infections which he studied. We have isolated this organism a great many times from cases of gaseous gangrene and infected wounds and have found that it has a definite number of characteristics by which it may be recognized.

A. MORPHOLOGY

It appears as a thick rod, the ends of which are blunt and slightly rounded. It is nonmotile and capsulated, and forms spores only under certain conditions of culture. It retains the Gram stain.

*Published by permission of the Surgeon General, U. S. Army.

Form and Dimensions.—In pathologic exudates or in very young cultures in broth, this bacillus is usually characterized by its short, thick forms. The ends of the bacillus are almost always slightly rounded, the angles being slightly bulging. Frequently the bacilli are grouped in pairs, the two elements forming a prolongation of one another or forming an angle of 45°. In older cultures long or curved forms are sometimes observed. We have encountered on several occasions, strains which developed curved bacilli even in young cultures in glucose broth and in liver peptone water medium. Some strains even form fairly long filaments, which are thicker and not as long as those seen with other anaerobic germs. Many variations in the thickness of the bacillus occur. While the majority of the bacilli studied had a breadth of 1.2 to 0.8 microns, it is not uncommon to see bacilli which are smaller and whose average breadth does not exceed 0.8 to 0.6 microns. These short forms usually showed extremities which were much more definitely rounded. The variations in form make the recognition of Bacillus welchii in stained smears difficult.

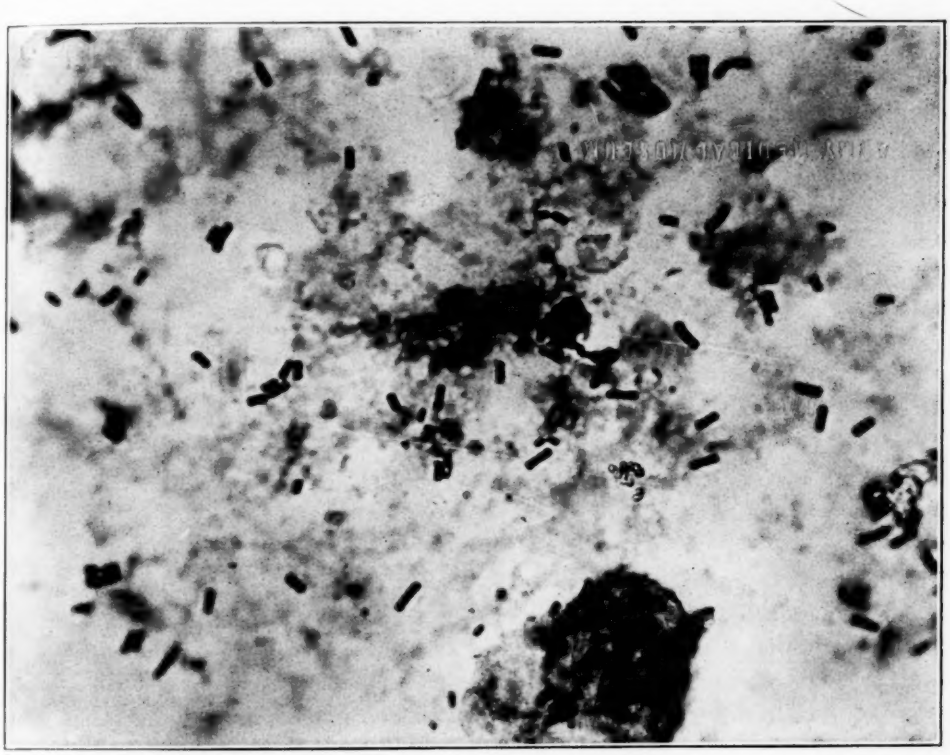


Fig. 1.—Baccillus welchii in muscle exudate. One sporulating bacillus close to center. (Army Medical Museum.)

Motility Flagella, Capsule.—The Bacillus welchii is definitely nonmotile. The capsule can be stained, using fuchsin ink as a mordant and staining with steaming carbol fuchsin, and is best demonstrated in young cultures grown on slant agar. Rarely bacilli from cultures in gelatin show capsules when stained with carbol gentian violet and exudates from animals dying of B. welchii infections show capsules fairly frequently.

Spores.—The question of sporulation of B. welchii has been studied extensively, but still remains a subject of considerable controversy. Spores never occur in media in which the bacteria produce acidity. It seems pretty definitely established that this germ can only sporulate in neutral or alkaline media. In serous exudates spores are usually not present. Cultures grown for more than a year in sugar-free broth containing white of egg fail to show spores. Some strains occasionally sporulate more or less rapidly in this medium; whereas certain other strains sporulate abundantly only on coagulated horse serum. Direct examination is often insufficient to demonstrate the presence of spores; they are very rarely seen in smears stained with Gram. They are stained with difficulty by the Ziehl method, and in many cases can only be demonstrated by the heating method.[13] They are seen rarely in the serous exudate of necrotic muscle, more especially with toxic strains of the Bacillus welchii. Fig. 1 shows one such spore amidst numerous vegetating bacilli. Spores are oval, occasionally attaining large dimensions, and they are almost always free. Where they are enclosed in the body of the bacillus, they are median or subterminal, and extend beyond the body of the bacillus. The spores of the B. welchii, as a rule, show little resistance to heat. It is known that they are usually killed by boiling. Serous exudates heated for one to three minutes at 100° after inoculation in broth tubes usually fail to grow B. welchii. At times it is possible to obtain cultures of this bacillus in broths inoculated with pathologic fluids that have been boiled for three to five and even eleven minutes, provided media have been heavily inoculated (Weinberg).

B. CULTURAL CHARACTERISTICS

The bacillus of Welch can be grown fairly easily in anaerobic media. For a long time it was thought necessary to have strictly anaerobic conditions, and many anaerobic apparatuses were suggested for the cultivation of this microorganism.

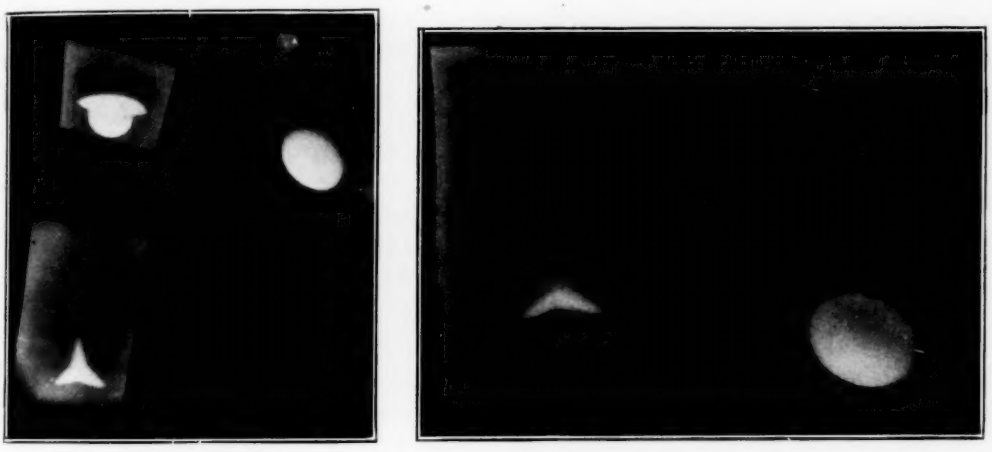
It was first shown by Tarozzi[14] that this organism could be cultivated in broth tubes containing sterile pieces of liver tissue, and later by Ori and Wrzosek[15] that the addition of sterile raw or autoclaved pieces of potato or other vegetable matter would also permit of anaerobic cultivation in what was formerly considered aerobic media. Douglas, Fleming and Colebrook[16] and later Wright and Fleming[17] showed that the Bacillus welchii could be cultivated in tubes of glucose broth containing a small quantity of asbestos wool, or even ordinary absorbent cotton, wool, platinum black, a rusty nail, and even a fine capillary tube containing diluted Welch bacillus culture. Besides these substances, cultures have been obtained by the addition of pieces of vegetable, such as potato, carrots, white beans, bread, cabbage, as well as cheese, sterile earth, dried and powdered albumin. All of these substances apparently create a nidus which protects inoculated bacilli against the effect of dissolved oxygen of the medium and give the organism its initial growth. As it grows it creates further anaerobic conditions. It is the most easily grown anaerobic organism of those met with in gaseous infections.

It does not grow as well in ordinary agar as in agar to which protein and carbohydrate have been added. The addition of blood or serum to ordinary agar

appreciably increases the vitality of the culture. Veillon's agar is a particularly good culture medium.

Veal Infusion Broth with 0.2 Per Cent Glucose.—Abundant growth with production of a large amount of gas. Clouds medium uniformly within the first 24 hours, and the culture settles slowly but progressively in several days at the bottom of the tube. The sediment packs down, and occasionally black pigment granules are present in the sediment. The culture has a butyric acid odor and the reaction is acid to litmus.

In *sugar-free broth,* to which a cube of coagulated egg white has been added, the same cultural characteristics are manifest. Egg albumin is slightly attacked at its margins or remains unaltered. After several months' incubation, it may become completely transparent, and is accompanied by a strong, disagreeable, nonputrid odor.



A. B.

Fig. 2.—Various types of colonies of B. welchii. A. Low magnification. B. High mignification. (Army Medical Museum.)

Liver Cube Plus Peptone Water.—Same cultural characteristics as above except that gas bubbles arise from liver and medium clouds within six to twelve hours. The liver cube turns brick red.

In *blood broth,* hemolysis occurs, the medium taking on a brownish tinge. There is a pronounced butyric acid odor.

Trypsinized Serum of Wright.—The bacillus grows well if sterile trypsin is added in 1/25 of its volume to normal serum.

Veillon Agar.—Development with marked production of gas and fragmentation of the agar. The colonies occasionally grow up to the water of condensation on the surface. Colonies have well-defined margins and are lenticular in shape. Certain variations in the forms of the colonies are often seen. Often they begin as "yellowish heart-shaped" forms and later develop into small "brioche-like" shapes, as the results of growths of small protuberances from the notches of the previously described heart-shaped forms. Fig. 2 shows the various shapes of the colonies of B. welchii. On the surface of agar, colonies appear round in

shape, with well-defined margins, grey in the center, and more transparent at the margins. Occasionally the lenticular colony develops a circular margin which has streaks radiating out toward the periphery, giving the colony an iris-like appearance. In glucose gelatin, colonies are of the same appearance as those seen in deep agar. Liquefaction of gelatin is slow and incomplete. It sometimes requires incubation over a period of months before the gelatin is liquefied to at least ⅓ or ¼ of its depth. Some strains liquefy gelatin completely after several months' growth.

On *blood agar* the colonies grow as well-defined, circular, slightly raised colonies with an opaque center which is granular and a periphery which is more translucent. The colony usually appears after 24 hours' incubation as small, opaque points and, after several days' growth, assumes typical colony shape. It is usually surrounded by a clear zone due to the hemolysis of the blood in the medium.

Deep Trypsin Agar.—B. welchii grows readily in this medium, producing a large amount of gas which fragments the medium, and forms lenticular colonies which grow up to within the water of condensation.

Andrade Veillon Agar.—The addition of 1 per cent solution of neutralized acid fuchsin to veillon agar inhibits somewhat the development of this bacillus. The colonies are less numerous and smaller, assume a light brick red color after several days' incubation, and the surrounding medium becomes tinged a light salmon color.

Litmus Milk.—Sometimes the litmus is reddened and sometimes it is completely decolorized. Coagulation occurs quickly en masse, owing to the large amount of acid produced. The clot then contracts, is riddled with gas bubbles, which gives it a sponge-like appearance, and floats on the clear whey. This reaction in milk is very characteristic and is spoken of as stormy fermentation. The milk has a butyric acid odor. This takes place within from 8 to 24 hours, provided fresh milk, which has been boiled and cooled previous to seeding, is used. Otherwise reaction is delayed for as long as 48 hours.

Coagulated Serum.—Very slightly attacked by some strains; usually is not changed at all, even after several days' incubation. Sporulation is at times very abundant in this medium. Swollen forms appear occasionally with free spores. Some bacilli staining with Gram often contain spores.

Meat Broth.—Culture is less abundant, media has a weak butyric acid odor, sporulation occurs at times, and meat assumes a brick red color.

Resistance.—Extremely feeble acidity corresponding to an H-ion concentration equal to that of a 1:30,000 N/HCl is sufficient to inhibit bacterial activity. (Wolf and Harris.)

Heat.—Heating above 90° is sufficient to stop the growth of the majority of strains.

C. BIOCHEMICAL PROPERTIES

1. *Saccharolytic Properties.*—The B. welchii contains a very powerful saccharolytic ferment and attacks carbohydrates, resulting in the production of gas, accompanied by a marked acidity. P. Simonds, who has studied twenty strains

derived from different sources, from this point of view, divides the B. welchii into four groups:

Group I ferments inulin and glycerin, resulting in the production of gas and acid, and no spores form in either of these media.

Group 2 ferments glycerin, with the production of gas and acid, but does not ferment inulin, and forms spores only in media containing inulin.

Group 3 ferments inulin, but not glycerin, and forms spores in media containing glycerin.

Group 4 ferments neither inulin nor glycerin and sporulates in both media.

This classification has been confirmed by H. Henry,[19] who has studied numerous strains of B. welchii isolated from war wounds. According to this author, B. welchii ferments constantly glucose, levulose, galactose, saccharose, maltose, raffinose, mannose, starch, dextrin and glycogen. Wolf and Harris have found that the B. welchii produces gas in media containing 3 to 4 per cent of lactose. The gas produced is 3.8 times the volume of the original medium. Gas is occasionally formed from certain amino acids which are glycogenetic. The gas is composed of hydrogen and carbon dioxide in the proportion of 2:1. Nitrogen has never been demonstrated in the gas that is formed. There is a latent period in gas production which lasts from four to eight hours when properly prepared medium is seeded with an active strain. Following this period there is a sudden drop in the production of gas, which seems due to a partial inhibition of the growth of the organisms by the products which are formed. There is then a secondary rise which reaches its maximum in about 24 hours.

During the latent period there is a slight rise in the H-ion concentration of the medium, and a maximum rise which corresponds with the production of acid during the sixth to the eighth hour.

The maximum gas production is attained when the H-ion concentration is that which corresponds to Ph = 4.52 to Ph = 4.56. When the acidity is increased, there is an inhibition of the metabolic activity of the microorganism.

The acids produced are 60 per cent volatile. These volatile acids consist chiefly of normal butyric acid varying from 5.4 grams to 8.3 grams per liter.

2. Proteolytic Properties.—The proteolytic properties of the B. welchii are still the subject of much discussion. It will liquefy gelatin slowly, will attack very feebly and slowly egg albumin, and does not seem to influence casein. Certain strains attack very slowly coagulated serum albumin. Tissier and Martelly consider the B. welchii an energetic proteolytic agent, particularly in sugar-containing media. In protein-containing media, the Bacillus welchii first forms amino acids before it begins to ferment carbohydrate substances. (Wolf and Harris.)

Peptolytic Power.—P. Simonds finds that it produces very little ammonia, even when inoculated into sugar broth, indicating that its power of breaking down noncoagulable nitrogen is negligible. Search for indol has given very contradictory results. The ability to produce indol seems to vary a great deal, depending upon the strains that are studied. In peptone water which contains 30 per cent of its nitrogen as amino acids, the Bacillus welchii forms, in addition, often as much as 20 milligrams of nitrogen contained in amino acid form. Ac-

cording to Wolf and Harris, the B. welchii is therefore peptolytic and, in media which contain only amino acids, it is also aminolytic. In media prepared from meat which is digested by trypsin and erepsin to the point where no biuret reacting substances are present, Bacillus welchii is still capable of metabolic activity, as shown by the increase in the amount of ammonia nitrogen and the reduction in the amount of amino acid nitrogen. According to Tissier and Martelly, the B. welchii secretes, in addition to a trypsin and an amylose, a fat-splitting ferment which saponifies fats.

D. SEROLOGIC CHARACTERISTICS

1. Hemolysins.—It secretes hemolysins for the blood corpuscles of man, rabbits, dogs, pigeons, white mice, hogs, cattle, sheep, horse, guinea pig, white rat, and hen. It dissolves hemoglobin out of the red cells of the guinea pig, rabbit, hen and pigeon. It destroys the red cells of the other animals. Young rabbits, dogs, and white mice show hemoglobinuria following injection of these substances. This hemolytic substance is destroyed by heating to 60° for one-half hour, prolonged incubation and exposure to light. It passes only partly through a Berkefeld filter.[22] It can be conserved in the ice chest. Cultures which have no hemolytic substances often contain hemagglutinins. Young cultures 15 to 16 hours in glucose broth contain an appreciable amount of hemolytic substances. One-tenth of a c.c. of total culture will hemolyze a dilution of red cells corresponding to 1/40 to 1/20 of a c.c. of packed red cells, 1.0 c.c. having therefore sufficient hemolysin to dissolve ¼ to ½ of a c.c. of packed red cells. Forty-eight hour cultures rapidly lose their hemolytic substances. It is neutralized by the serum of a large number of animals. Horses have normally an appreciable amount of antihemolysin. White mice are very susceptible to the Welch hemolysin.

2. *Agglutinins.*—It has been possible to produce agglutinating substances by the injection of massive amounts of culture into rabbits and horses. The best agglutinating serum agglutinates its homologous strain in 1/500 and 1/2000 dilution, whereas a serum furnished us by Dr. Weinberg agglutinated a strain isolated from an infected war wound in a dilution of 1-800. A good agglutinating serum may be prepared by the injection either of washed bacilli or bacillary emulsions detoxicated by the addition of ⅓ of its volume of Lugol's solution.

3. *Precipitins.*—McCampbell[23] produced precipitins in the serum of rabbits, and Korentchewsky[24] claims to have produced precipitins in the serum of animals injected per rectum and fed with filtrates from cultures of B. welchii.

4. *Bacteriolysins.*—McCampbell claimed to have produced bacteriolysins against the B. welchii. In 1915, we succeeded in producing bacteriolysins in a horse injected with small quantities of killed and living cultures of the B. welchii which had a bacteriolytic titer of 1:100 and ½ c.c. of which protected against a lethal quantity of a 24 hour culture.

5. *Complement-Fixing Bodies.*—McCampbell found complement-fixing bodies in the serum of animals injected with B. welchii. Korentchewsky claimed similarly to have found complement-fixing bodies in the serum of dogs fed with culture filtrates. Rocchi[25] did not obtain uniform results.

6. *Toxins* have been isolated from cultures and are best separated from 18 to 24 hour cultures of the bacillus in glucose broth. Bull[26] has suggested the use of broth to which fresh pieces of rabbit muscle are added, whereas De Kruif[27] has suggested the addition of ⅕ volume of horse serum. Antitoxins have also been prepared, but their method of preparation and titration will be more fully discussed in a subsequent paper.

D. PATHOGENIC POWER

The pathogenicity of the B. welchii is extremely variable, as is that of all the anaerobic bacteria encountered in gaseous gangrene. Strains isolated from fatal cases of gaseous gangrene are usually very pathogenic for the guinea pig, and are able to retain their virulence over a period of months. The pathogenic power is titrated by injecting 24 hour cultures of the bacillus in 0.2 per cent glucose broth into the muscles of the thigh of a guinea pig.

One-fourth to ⅛ of a c.c. of 24-hour culture in glucose broth of the most pathogenic strains kills a guinea pig in 24 to 48 hours. The best strain studied at the Central Medical Department Laboratory of the A. E. F., killed a 350 gram guinea pig in less than 20 hours following injection of $\frac{1}{10}$ of a c.c. of a 20-hour culture in broth.

By separating the toxin from young cultures either by centrifugation or filtration through Berkefeld or Mandler filters, it is possible to obtain a toxin which will kill a guinea pig following intravenous injection of ½ to 1.0 c.c. within five minutes.

The study of experimental infection with B. welchii has thrown a great deal of light on the pathogeny of gas gangrene. For a long time there was a great deal of discussion regarding this point. It was thought by many that B. welchii was always capable of producing so-called gas bacillus infection. Then Marbais[28] and others found that injection of large quantities of washed bacilli failed to produce any lesions whatsoever in both guinea pigs and monkeys. Other investigators then proved that lesions could be produced if lactic acid or powdered glass was injected with the bacilli, or if a slight injury to muscles preceded the injection.[29]

De Kruif[30] showed that the toxin of the bacillus exercised a very definite aggressive action on the bacilli. Washed bacilli in relatively enormous quantities produced no or very slight injury, whereas the addition of minimal quantities of toxin would produce rapidly a very fatal result.

The symptoms following injection of bacilli plus toxin or after injury to muscles are invariably the same. If culture is virulent, it is not uncommon to see swelling and induration of the region injected within four to six hours after inoculation. The swelling increases and in about six to eight hours crackling is perceptible. The swelling increases, and extends up to the abdomen. The animal holds its limb in a flexed position to relieve the tension of the tissues. Swelling is due chiefly to edema, and to gas infiltration. As the bacteria multiply, the symptoms of intoxication become manifest and are the same as those observed when sublethal doses of toxin are injected into the circulation of sus-

ceptible animals. They act on the nervous system, producing tremor, slight convulsion, hiccup, bristling of the hair, constipation by paralysis of the bowel. They affect the circulatory system as evidenced by the extreme rapidity of the pulse.

Before death, paralysis of the hind legs occurs and the animal refuses food. It remains hunched up and motionless, and its respirations are markedly increased. Death intervenes by respiratory failure rather than by cardiac paralysis.

The lesions are those referable to the muscles, the blood vessels and the fat of the subcutaneous connective tissue. The muscles are first rendered necrotic by contact with the toxin which is a protoplasmic poison, and this has been shown experimentally *in vitro* by K. Taylor[21]. The carbohydrates of the necrotic muscle are then attacked in turn by the saccharolytic ferments of the bacilli which produce gas and acid. The gas infiltration results from this change. The edema which occurs has been ascribed by some authors to the acid produced,[19] whereas others maintain that it is due to the pressure of the infiltrating gas.

The toxin seems to exert a specific effect on the muscle fibers of the media coat of the blood vessels, with a tendency to rupture of the blood vessels and consequent hemorrhage. The exuded blood is hemolyzed by the hemolytic substances secreted by the B. welchii. This diffusion of laked blood is particularly noticeable with toxic strains.

REFERENCES

[1]Simonds: Studies in Bacillus Welchii with Special Reference to Classification and Its Relation to Diarrhea. Monograph No. 5, Rockefeller Institute for Medical Research, Sept., 1915.
[2]Achalme: Compt. rend. Soc. de Biol., July 25, 1891, pp. 651-656.
[3]Welch and Nuttall: Bull. Johns Hopkins Hosp., 1892, iii, 81-91.
[4]Fraenkel: Centralbl. f. Bakteriol., 1893, xiii, 13-16.
[5]Veillon and Zuber: Arch. d. Med. exper., 1897, x. 517-545.
[6]Migula: System of Bakterien, 1900, Jena, Fischer.
[7]Fraenkel: Ueber Gasphlegmone, 1893, Hamburg and Leipzig.
[8]Guillemot: Compt. rend. de la Soc. de Biol., Nov. 5, 1898, pp. 1017-1019.
[9]Hitschmann and Lindenthal: Sitzungsberichte der Kaiserlich Akademie der Wissentchafter in Wien, 1899, cviii, III Abt., pp. 67-239.
[10]Welch: Bull. Johns Hopkins Hosp., 1900, ii, 185-204.
[11]Albrecht: Arch. f. klin. Chir., 1902, lxvii, No. 3.
[12]Kamen: Centralbl. f. Bakt., I Abt. Orig., 1904, xxxv, 554-563, 686-712.
[13]Weinberg and Seguin: La Gangrene Gazeuse, Paris, 1918.
[14]Tarrozzi: Centralbl. f. Bakteriol., Abt. I, Orig., 1905, xxxviii, 619.
[15]Wrzosek: Centralbl. f. Bakteriol., Abt. I, Orig., 1907, xliii, 17.
[16]Douglas, Fleming, and Colebrook: Studies in Wound Infections, Growth of Anaerobic Bacilli in Fluid Media, Lancet, London, 1917, ii, 530-532.
[17]Wright, Fleming, and Colebrook: Sterilization of Wounds by Physical Agencies, Lancet, London, June 15, 1918.
[18]Wright and Fleming: Acidemia in Gas Gangrene, Lancet, London, Feb. 9, 1918.
[19]Henry, H.: Investigation of Cultural Reactions of Certain Anaerobes Found in Wounds, Jour. Path. and Bacteriol., xxi, 376.
[20]Wolf and Harris: Biochemistry of Pathogenic Anaerobes, ibid., xxi, 386.
[21]Tissier and Martelly: Recherches sur la Putrefaction de la Viande de Boucherie, Ann. de l'Inst. Pasteur, 1902, xvii, 540.
[22]Ouranoff, A.: Sur l'hemotoxine du B. Welchii (B. perfringens), Compt. rend. Soc. de Biol., 1903, 1917, lxxx, 706-708.
[23]McCampbell, E. F.: Toxic and Antigenic Properties of B. Welchii, Jour. Infect. Dis., 1909, vi, 537.
[24]Korentchewsky: Etude biologique du B. perfringens et du B. putritieus, Ann. de l'Inst. Pasteur, 1909, xxiii, 91-95.

[25]Rocchi: Centralbl. f. Bakteriol., I Abt. Orig., 1911, lx, 579-581.
[26]Bull and Pritchett: Jour. Exper. Med., July, 1917, pp. 119-138.
[27]De Kruif: Verbal communication.
[28]Marbais, C. R.: Soc. de Biol., 1914.
[29]Jablons: Pathology of War Surgery, Jour. Am. Med., Assn., July, 1915.
[30]De Kruif and Bollman: Mechanism of Infection with B. Welchii, Jour. Infect. Dis., 1917, xxi, No. 6, pp. 588-599.
[31]Taylor, K.: Factors Responsible for Gaseous Gangrene, Lancet, London, January 15, 1916, pp. 123-125.

LABORATORY METHODS

A METHOD FOR THE COLLECTION OF URINE FROM EACH KIDNEY SEPARATELY IN THE DOG*

By F. S. Hopkins, M.D., and W. C. Quinby, M.D., Boston, Mass.

In the conduction of certain types of experimental investigation it is important to be able to obtain the product of each kidney separately, and especially is this importance noted in work dealing with experimental nephritis. Of course, in those cases where the experiment can be performed as an acute one, lasting at the most only a few days, it is quite feasible to collect the urine from each ureter, which has been provisionally delivered from the abdomen onto the surface of the skin in the loin. For prolonged observation, however, this method is not feasible on account of the unavoidable advent of infection which soon manifests itself and leads to definite pyelonephritis and death.

In the female dog it is possible to collect urine separately from the bladder by means of the cystoscope, but this method also has very definite limitations. In the first place one must be very adept at cystoscopic technic, because the use of this instrument in the dog's bladder is made more difficult than it is found to be in the human bladder on account of the fact that in the dog this viscus is practically an intraperitoneal organ, and therefore very movable. Furthermore, the dog's ureter, on leaving the bladder, takes a downward and sometimes a downward and forward course for approximately 1 cm., thus making the introduction of a ureteral catheter very difficult, but, as mentioned above, ureteral catheterization is possible in the dog, although it can not be performed widely and also can not be performed frequently because of the extreme sensitiveness of the bladder mucosa in this animal. This latter fact leads to hyperemia and edema about the ureteric orifice which has been catheterized, thus making the identification of the orifice at a second period quite difficult.

A further method of segregation of the dog's urine, and one which we believe to be superior to any other, is made possible by the use of the uterus as a receptacle of the products of one kidney while the normal relations between the bladder and other kidney are maintained. As will be seen from the diagram this is not an especially difficult procedure. Its description is as follows: By laparotomy the ureter on one side is delivered from behind the peritoneum and cut across at a convenient point above the bladder; the stump remaining attached to the bladder is tied off. The corresponding horn of the uterus is then incised by a small longitudinal cut, and an anastomosis is made between the ureter and uterus. This

*From the Laboratory of Surgical Research, Harvard Medical School, Boston.

Reported at the tenth annual meeting of the American Society for Clinical Investigation, Atlantic City, June, 1918.

union is made much easier by using as a splint a small No. 5 ureteral catheter. This is first passed up the ureter toward the kidney for a sufficient distance and then its lower end passed in through the incision in the uterus until it appears at the vulva. The ureter is then stitched to the uterus with a few interrupted stitches of blood-vessel silk. The cornu of the uterus above the point of anastomosis is then ligated in order to prevent the extension of urine in an upward direction, and similarly the opposite cornu is then closed and the catheter appearing through the

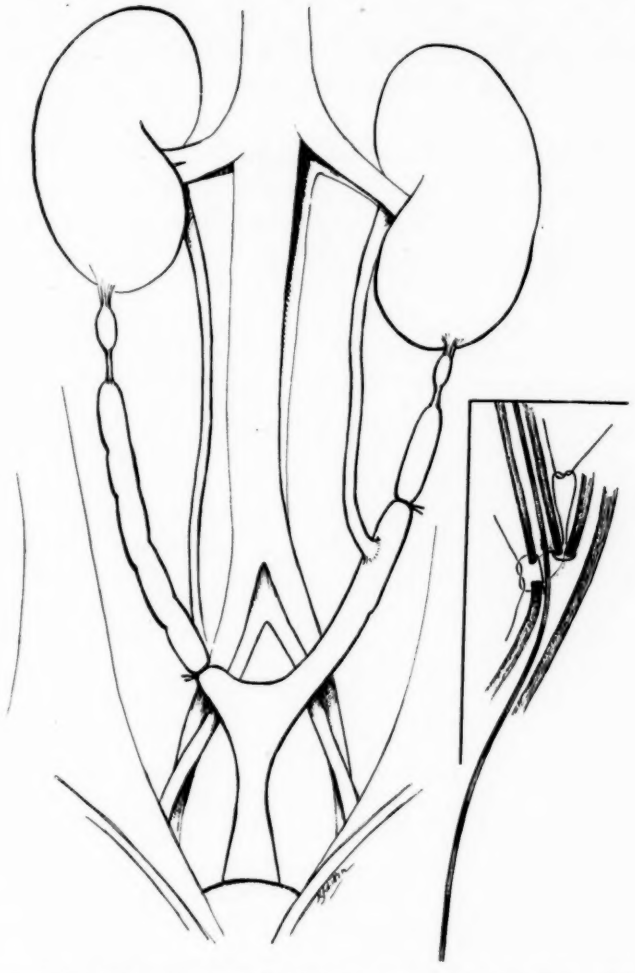


Fig. 1.—Semidiagrammatic sketch of anastomosis between ureter and left uterine cornu. Insert shows detail of anastomosis using ureteral catheter as splint.

vulva is cut off so as to be inaccessible to the dog. If it be intended to prolong the observations over a considerable period of time, it is easy to avoid the complications which might be caused by the advent of catamenia by the removal of the ovaries at the time the anastomosis is made.

We thus have established a condition in which the urine drains through the catheter into the vagina for a period of approximately 48 hours, after which the catheter can be safely withdrawn by forceps introduced into the vulva. At the

end of one week it is possible to collect the products of each kidney in the following fashion: A glass tube is adjusted so as to fill the vulva and with this in place collection can be made over the required time period. The urine from the bladder is subsequently withdrawn by catheterization through the urethra. Analysis of the urine collected by this uterovaginal route shows it to be normal and similar to that from the other side, with the exception that it is apt to contain an excess of mucus.

SOME LIMITATIONS OF THE FLOTATION METHOD OF FECAL EXAMINATION*

By J. Daley McDonald, M.S., Berkeley, Cal.

THE brine flotation-loop method of fecal examination for intestinal worms was first employed by Kofoid and Barber[1] in the examination of troops of the United States Army.

This method is based on the fact that the specific gravity of the ova of the worms is considerably less than that of a saturated solution of sodium chloride. Barring interfering factors, this will result in the rise of the ova to the surface of the brine mixture of feces, while the heavy and the penetrable material will settle, thus producing a marked segregation. The authors give the following account of the manner of procedure.

"The method finally perfected by us may be designated as the brine flotation-loop method. It consists in mixing a large fecal sample thoroughly in concentrated brine, in a paraffined paper can of from two to three ounces capacity, forcing the coarse float below the surface by means of a disk of No. 0 steel wool, and then allowing the can to stand one hour for the ova to ascend. The surface film is then looped off with wire loops, one-half inch in diameter, and examined on a slide without a cover glass." [Page 1558.]

This method was adopted for making routine fecal examinations by the Division of Parasitology of the California State Board of Health in January, 1919. Its advantages over other methods in matters of rapidity, simplicity, and adaptability have already been demonstrated beyond doubt. During the first half of the year several hundred Orientals (residents of California) were examined both for intestinal worms and for intestinal protozoa. A small portion of each fecal specimen was set aside for the protozoan determination and the remainder examined by the flotation method as just described. Early in this work the ova of *Clonorchis sinensis* were found in the smears being examined for protozoa, while in the portion of the identical specimen, on which the flotation method had been previously employed, not a single ovum had been found. In the next 500 specimens examined, ten cases of *clonorchiasis* were diagnosed by means of the method used for protozoa. An equal number must have passed undetected, for the method of examination for protozoa necessitates the use of an extremely small sample of feces and permits little or no concentration. From the same 500 specimens no ova of *Clonorchis* were found by the flotation method. These re-

*Contribution from the Division of Parasitology of the California State Board of Health, Berkeley, Cal.

sults subject to question the effectiveness of this method for *Clonorchis sinensis*, and the following experiments were undertaken with the hope of eliminating this element of uncertainty.

The first test consisted in making a careful comparison of the flotation and the centrifuge methods, using for this purpose forty specimens among which there was likelihood of finding some cases of *clonorchiasis*. The specimens were divided, furnishing a part for each method. The procedure followed in the centrifuge method was that employed in the United States Immigration Station Hospital, Angel Island, California, and described by Billings and Hickey.[2] The results of this comparative test may be found in Table I. From these results it will be seen that the flotation method is more critical than the centrifuge method

TABLE I

COMPARATIVE RESULTS IN DETECTION OF OVA IN FORTY SPECIMENS EXAMINED BY BOTH THE CENTRIFUGE AND THE FLOTATION METHODS

POSITIVE FOR	NUMBER OF CASES DETECTED BY THE CENTRIFUGE METHOD	NUMBER OF CASES DETECTED BY THE FLOTATION METHOD
Trichuris trichiura	13	15
Ascaris lumbricoides	10	11
Hookworm	3	5
Clonorchis sinensis	3	0

for the detection of the ova of the three nematodes found, but it completely failed in the detection of the ova of the trematode, *Clonorchis sinensis*. At the conclusion of the experiment, the sediment remaining in the containers in which the brine had been used, was examined. A large number of ova of *Clonorchis* were present in each of the three cases in which their presence was previously shown by the centrifuge method. The ova had been present, but had not risen to the surface of the brine emulsion.

There seemed a possibility that the age of the ova or the length of time allowed for flotation might be factors in their failure to rise. Some evidence regarding these matters was furnished by experience in the routine examinations in the laboratory. The 500 samples among which the ten cases of *clonorchiasis* were found had been sent to the laboratory in several different lots and had been from one to four days in transit. The specimens used in making the comparison of the centrifuge and flotation methods had been voided within the previous nine hours. These two considerations would indicate that variation in the age of the ova from the time of deposition up to four days following makes no appreciable difference. The time elapsing between the mixing of the specimen and the looping off of the surface, i.e., the flotation time, often varies from forty-five minutes to one hour and fifteen minutes in the making of routine examinations, due to frequent interruption preventing the looping off of the surface at the exact expiration of one hour. So, within these limits it was already evident that the flotation time was of no consequence.

A few simple laboratory tests of a corroborative nature were made possible through the kindness of Dr. J. P. Hickey of the United States Immigration Station, Angel Island, California, who supplied material containing an unusually large number of the ova of *Clonorchis sinensis* and some ova of *Trichuris trichiura*. The first use of this material was made about twenty-four hours after

being voided. For the first two experiments (A and B) it was mixed with feces containing ova of the sort recently described by Kofoid[3] as those of a new parasitic nematode of man, *Oxyuris incognita*. These ova, with those of *Trichuris* already in the original material, served as a control on the method. Experiments A and B were identical, being intended as a check, each against the other. In conducting the experiments care was taken to follow closely the description by Kofoid and Barber. Paraffined 2-oz. drug cans served as containers and No. 0 steel wool was used for filtration. A period of fifteen minutes was allowed to elapse before looping off the surface for the first examination. Previous experience had shown this to be the minimum time during which diffusion currents would cease and permit any appreciable stratification to take place. Sampling before such stratification had occurred would be equivalent to mixing the material in water merely, and removing a drop for examination while the material was still in suspension. Following the first examination, the surface was looped off and examined at fifteen minute intervals up to one hour and then at thirty minute intervals until two hours after mixing. This procedure was much simpler than preparing a special sample for each period. Besides, since care was taken to make sure that the entire surface was removed each time, the number of ova found at each examination represented almost exclusively those which had risen during the preceding interval. As a result some evidence was gained regarding the most opportune time for looping off the surface when making routine examinations. This manner of conducting the experiments must be borne in mind when referring to Table II, for in the usual procedure the number of ova

TABLE II

RESULTS OF EXPERIMENTS TESTING THE EFFECTIVENESS OF THE BRINE FLOTATION-LOOP METHOD IN THE DETECTION OF OPERCULATED OVA

FLOTATION TIME	EXPERIMENT A. AGE OF OVA 24 HRS.	EXPERIMENT B. AGE OF OVA 24 HRS.	EXPERIMENT C. AGE OF OVA 24 HRS.	EXPERIMENT D. AGE OF OVA 48 HRS.	EXPERIMENT E. AGE OF OVA 72 HRS.	Experiment with the ova of Pneumonoeces similiplexus. Age of ova, 48 hrs.	Experiment with the ova of Fasciola hepatica. Age of ova, 12 hrs.
The numbers represent the ova detected at each interval.	Clonorchis sinensis Trichuris trichiura Oxyuris incognita	Clonorchis sinensis Trichuris trichiura Oxyuris incognita	Clonorchis sinensis Trichuris trichiura	Clonorchis sinensis Trichuris trichiura	Clonorchis sinensis Trichuris trichiura		
15 min.	0 2 0	0 2 1	0 0	0 1	0 0	0*	0
30 min.	0 1 2	0 3 0	0 0	0 3	0* 0	0*	0
45 min.	0 2 1	0 0 3	0 1	0 2	0* 0	0*	0
1 hr.	0 1 0	0 1 1	0 0	0* 3	0 0	0*	0
1½ hrs.	0 0 1	0 1 0	0 2	0 4	0 0	0	0
2 hrs.	0 0 0	0 0 0	0 3	0 1	– –	–	–
No. of ova under 22 mm. coverglass of sediment	216 — —	180 — —	680 —	600 to 700	600 to 700	Many distorted	1200

*In these examinations two or three ova were found, not on the surface of the drop, but in the sediment which had settled on the slide.

reported at one hour would be the sum of those reported in this table for the 15, 30, 45 minute and one hour intervals. Experiments C, D, and E were identical with A and B, except that the ova used were progressively older. Table II gives in detail the results for each experiment at the stated intervals.

It will be noted from Table II that not a single ovum of *Clonorchis* was detected by the flotation method. That they were present in enormous numbers is proved by the counts made of the number to be found in a drop of the sediment under a single 22 mm. square cover glass. The lesser number of ova in the sediment in experiments A and B is due to the fact that the material used was mixed with a more than equal quantity of feces not containing the ova.

There are two obvious differences between the ova of *Clonorchis* and those of the other parasites in the detection of which the flotation method has proved so effective. First, the ova have an operculum, characteristic of the ova of most of the trematodes (excluding, of course, Schistosoma). Second, they are of very small size, varying from 23 to 27 microns long by 11 to 14 microns broad. This led to the trial of the method on the operculate ova of two other trematodes. The ova first used were not from a human parasite but from *Pneumonoeces similiplexus*, the lung-fluke of frogs and salamanders. This form was chosen because the material was readily available and because the ova are approximately the size of those of *Clonorchis*, though having a somewhat thinner shell. The ova from two of these flukes, which had been deposited in water, were mixed with human feces in considerable concentration. The remainder of the experiment was conducted the same as those described above. No ova were detected, though many were found in the sediment (see Table II).

Other operculate ova used were those of *Fasciola hepatica*. These were obtained from infected cattle, and were mixed with human feces. The material was then manipulated in the same manner as in the previous experiments. None of these ova were floated to the surface during the entire period of two hours. The concentration of the ova in the material used is indicated by the number found in the sediment after the use of the brine (see Table II).

In most cases, after examining the surface of the drop on the slide, the excess fluid was removed, leaving any sediment which might have settled through the drop. Examination of this sediment in each of three cases revealed two ova of *Clonorchis* and in several instances two or three ova of *Pneumonoeces* were found. These instances are marked by an asterisk in Table II. The presence of these ova in the material looped off is probably explainable by their adherence to debris. Should an ovum become attached to a bit of debris which subsequently floated to the surface, then the ovum would be carried up with it. Both would then be looped off onto the slide. In routine manipulation the wire loop is usually struck sharply against the slide in order to break and release the film which it has transferred. This concussion would in all probability loosen the ovum from the debris, whereupon the former would settle to the bottom of the drop on the slide and completely escape notice in an examination of the surface of the drop. Only the surface of the drop is supposed to be examined in regular procedure.[4]

This explanation is strengthened by the fact that the largest and heaviest ova, those of *Fasciola hepatica* were never found, the next in size and weight, those of *Clonorchis sinensis* were found in only ten per cent of examinations,

while the smallest and lightest, those of *Pneumonoeces similiplexus,* were found in the sediment on the slide in eighty per cent of the examinations. This behavior would be logically expected, for small light ova would be carried much more readily than heavy ova by adhering to bits of debris.

DISCUSSION

Kofoid and Barber in their original description of the brine flotation-loop method call attention to its ineffectiveness in the detection of infection with Strongyloides.[5] In the same paper the authors make a tabulation showing the comparative efficiency of this method and the centrifuge method, but in it no ova of trematodes, in fact no operculate ova of any sort, are recorded. However, the following statement occurs somewhat further along. "The ova of parasites such as * * * and of trematodes are floated up by the brine into the surface layer of the pool without distortion or noticeable change in appearance during the usual period of examination" (page 1559). The results reported in this paper make it necessary to qualify this statement with regard to the effectiveness of the flotation method for the ova of trematodes, and subject to question its effectiveness in the case of all operculate ova. In the report made by Kofoid, et al,[6] of the occurrence of intestinal parasites in troops of the United States Army, of a total of 1500 examinations made by this method only one operculate ovum was detected, viz., that of *Diphyllobothrium latum.*[7] However, this is in all probability due to the fact that those parasites which produce operculate ova are very rare in those who have not resided or traveled much outside of the United States.

In the above experiments the failure of the ova to float to the surface of the brine could scarcely be due to their density, for all of them have comparatively thin shells and are probably of less specific gravity than the ova of *Ascaris* or *Trichuris.* It may perhaps be explained by the permeability of the ova. Stephens[8] gives an account of certain yolk granules, not always used up during the development of the embryo, which he believes must serve some other purpose than food supply, possibly that of imbibing water for the use of the embryo. If this is the case then it may be that the brine penetrates the ova of trematodes. But the penetration must needs be very rapid for examinations were made in each experiment within fifteen minutes after addition of the brine. No other direct evidence regarding this hypothesis was gained from these experiments.

A seemingly more plausible explanation of the failure of the flotation method is suggested by the condition of the ova following their treatment with the brine. Invariably, following the use of the brine, ova were found, in which the operculum had become separated from the rest of the shell. Of the ova found in this condition the proportion varies from fifteen per cent in *Pneumonoeces* to fifty per cent in *Clonorchis.* In those ova in which the operculum had not actually opened, the line of attachment was markedly more conspicuous, than in untreated ova, suggesting that it had been loosened. The loosening is probably caused by either osmotic pressure or the variation in pressure between the medium and the contents of the ovum. Apparently the stage of development made no difference in the opening of the operculum, for it occurred in both *Clonorchis* and *Fasciola.* In the former the content of the ovum is a nearly

completely developed miracidium while in the latter the embryo is in the early cleavage stages only. Since the efficacy of the flotation method is dependent upon the lighter specific gravity of the ovum as compared with the brine, the loosening of the operculum and consequent entrance of the brine into the ovum defeats the purpose of the method.

The brine flotation-loop method has many advantages over other methods in the saving of time and materials and in the ease of manipulation. However, as shown by these experiments its range of application is limited by the type of ovum. Infections with *Clonorchis* and *Fasciola* are not detected by this method and its effectiveness in the detection of any operculate ova is very doubtful.

REFERENCES

[1]Kofoid, C. A., and Barber, M. A.: Rapid Method for Detection of Ova of Intestinal Parasites in Human Stools, Jour. Am. Med. Assn., Nov. 9, 1918, lxxi, 1557-1561.

[2]Billings, W. C., and Hickey, J. P.: Some Points about Hookworm Disease, Its Diagnosis and Treatment, Jour. Am. Med. Assn., Dec. 23, 1916, lxvii, 1908-1912.

[3]Kofoid, C. A., and White, A. W.: A New Nematode Infection of Man, Jour. Am. Med. Assn., Feb. 22, 1919, lxxii, 567-569.

[4]Kofoid, C. A., and Barber, M. A.: Jour. Am. Med. Assn., Nov. 9, 1918, lxxi, 1559. Note also diagram, page 49, Lab. Methods of U. S. Army, Med. War Manual No. 6, Philadelphia, 1919, Lea and Febiger.

[5]Kofoid, C. A., and Barber, M. A.: Jour. Am. Med. Assn., Nov. 9, 1918, lxxi, 1557.

[6]Kofoid, C. A., et al: Intestinal Parasites in Overseas and Home Service Troops of the U. S. Army, Jour. Am. Med. Assn., June 14, 1919, lxxii, 1721-1724.

[7]This generic name takes precedence over Bothriocephalus, as shown by Magath: The Eggs of *Diphyllobothrium latum*, Jour. Am. Med. Assn., July 12, 1919, lxxiii, 85-87.

[8]Fantham, H. B., Stephens, J. W. W., and Theobald, T. V.: The Animal Parasites of Man, 1916, New York, Wm. Wood and Co., footnote on page 223.

The Journal of Laboratory and Clinical Medicine

VOL. V. MARCH, 1920 No. 6.

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Ann Arbor, Mich.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
HANS ZINSSER, M.D. - - - NEW YORK
PAUL G. WOOLLEY, M.D. - - DETROIT
FREDERICK P. GAY, M.D. - - BERKELEY, CAL.
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
WARREN T. VAUGHAN, M.D. - . BOSTON

Contents of this Journal Copyright, 1920, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

The Science of Ventilation and Open Air Treatment

THE well-being of a conscious animal in relation to its environment constitutes the main problem of the study of ventilation. In the case of animals leading an outdoor life, it is a problem of relatively little importance, but for those like man which spend much of their time in confined spaces, it is a problem of great importance, for in them it becomes necessary to determine the limits within which the outside influences may be altered without detriment to health or comfort.

It is often imagined that ventilation is a matter merely of pure air and that it therefore becomes a problem requiring attention only in cases where the air has been polluted by the crowding together of many people. This attitude is a wrong one, for there is very good evidence to show that much discomfort and ill health could be avoided if people understood more clearly the physical conditions of the atmosphere which bear a relationship to the well-being of the body.

When our knowledge of the function of breathing became developed to the extent of showing that an animal requires the oxygen of the air for the living processes of its body, and as a result of these processes that it produces carbonic acid, which is then added to the air, it was natural to suppose that the

unfavorable effect of overcrowded confined spaces was due either to the using up of the available oxygen or to a poisonous action of the carbonic acid.

It needs only a few words to point out how utterly erroneous were these earlier explanations. That deficiency of oxygen is no factor is indicated by the facts, first, that this gas is seldom reduced by more than one per cent, even in the most crowded places; and secondly, that people live a normal existence at altitudes at which the oxygen percentage, measured at sea level, is reduced to less than two-thirds the normal.

It is not altogether easy to understand why excess of CO_2 was thought to be responsible for the evil effects of vitiated atmospheres. No doubt the chief reason was that the percentage of this gas is often raised in such atmospheres, but this is nothing more than coincidence, for on the one hand most unsuitable conditions may exist when the percentage of CO_2 is normal, and on the other, air loaded with almost a hundred times the percentage found even in the most polluted atmosphere can be breathed for indefinite periods of time without any unfavorable symptoms.

As a matter of fact, even in the open, we are constantly taking into the pulmonary alveoli large percentages of CO_2, for obviously with each inspiration the first air to be drawn in is that which remains over in the air passage from the preceding expiration. This air contains somewhere about 5 per cent of CO_2, and in quiet breathing it amounts in volume to about one-third of all the air that is drawn in from the outside. This in itself indicates that CO_2 *per se* can not be poisonous, and when we consider further the now well-known fact that a certain amount of this gas in the alveoli is absolutely essential to the well-being of the animal, the whole hypothesis of its toxic action becomes, to say the least of it, absurd. Indeed so important is the presence of this constant amount of CO_2 in the alveolar air that whenever there comes to be a marked increase in the amount of CO_2 in the atmosphere, the breathing becomes greater, so as to ventilate the air sacs more thoroughly, and thus keep the relative amount of CO_2 in them at the normal level. The extent of this increase in respiration is usually so small as to be unnoticed by the individual, and certainly increased breathing is not one of the symptoms of which persons complain who are living in polluted atmospheres.

In face of such evidence, even the most ardent supporters of the theory that the vitiated air owes its evil influence to CO_2, were compelled to abandon their position, but they did not do so without a final attempt to retain for determinations of CO_2 a certain significance in the appraisement of the healthfulness of air. Their new interpretation was to the effect that the CO_2 percentage is proportional to the amount of deleterious organic matter, and for many years this view prevailed. It is still believed by some that an increase from the normal of 3 to 10 parts of CO_2 per 10,000 parts of air indicates a degree of organic pollution which is dangerous to health. More recent work definitely shows, however, that this view also must be abandoned, and there remains for CO_2-analysis only the secondary value that it indicates, in a readily measurable way, to what extent the inside air is being mixed by ventilation with pure air from the outside. However free this dilution may be, the atmosphere may still be deleterious to health and comfort unless certain other properties of it are incidentally altered.

This interpretation of the value of CO_2 analysis naturally leads to a consideration of the next possibility, namely, that the air in confined spaces is contaminated by the accumulation of organic poisons derived from the exhaled air of the persons living in it. It is many years ago now since experiments apparently proving this hypothesis were published. These have been shown to be entirely fallacious, and we need refer to only one group of them here, namely, those that were devised to show that inhalation by one animal of volatile proteins contained in the exhaled air of others caused anaphylactic reactions. As proof for this hypothesis, experiments were performed in which a man breathed through a filter of glass wool (to catch any saliva) into a cooled vessel, and the condensed vapor was then inoculated in appropriate dosage into guinea pigs, so as to sensitize them, and a month or so later the animals were inoculated with a minute trace of human blood serum. The injected animal showed decided symptoms of anaphylactic shock, whereas other animals not previously sensitized were unaffected by the injection of the same amount of serum. Such results taken by themselves did seem to afford substantial support for the new hypothesis, but it is almost certain that they depended on contamination of the condensed vapor by traces of saliva which it is impossible to keep out by any kind of filter. This saliva contains traces of soluble protein (mucin) which had been responsible for the anaphylactic reaction. The symptoms are, however, entirely dissimilar from those of a vitiated atmosphere. Hay fever, some forms of asthma, and the reaction which some persons show when near to horses may be due to anaphylaxis, but the symptoms are not at all like those of persons breathing polluted air.

Once and for all, the toxic theory, as we may call it, both in its new and its old form, is disproved by a very simple series of experiments performed a few years ago by Leonard Hill, Flack and others.[1] These observers kept rats and guinea pigs in deep boxes so that they were huddled together in a very poorly ventilated place, the atmosphere of which indeed often contained 1 per cent of CO_2—ten times more than the legal limit. The animals lived and thrived for months, although they must have been breathing air which was highly contaminated by the supposed volatile proteins. Not only did the animals show no symptoms while in the box, but they failed to exhibit any anaphylactic reaction when, after some time, they were inoculated subcutaneously with the serum of animals of the other species with whom they had been in cohabitation. This was really a most excellent test of the anaphylactic theory because there are probably no two animals in which anaphylaxis is more pronounced than in the rat and guinea pig. The only things that were found to be of importance in maintaining the animals in a thriving condition were cleanliness and plenty of food.

By an eliminative process we are gradually approaching the correct solution of our problem, but before we proceed to consider this, it may be well to remark that the odor of polluted air has nothing whatever to do with its unhealthy influence, except in so far as it excites disgust and puts one off his appetite. Indeed one very soon becomes so accustomed to these odors that they fail entirely to be sensed after a short period in contact with them. Their influence is entirely psychologic. In many trades and occupations people are constantly exposed to odors that are almost unbearable to one who is unused to them, and these people are perfectly healthy, and indeed do not complain at all of the smells.

We have so far considered in what is approximately their chronologic order the various hypotheses that have been brought forward to account for the harmful influence of vitiated atmospheres. We have done this mainly in order to correct any false conclusions that may still exist in connection with the subject.

And if further evidence be demanded to justify this position, there is one crucial experiment which once and for all shows that changes in the chemical composition of the atmosphere have no relationship whatsoever to the unhealthful influence of vitiated air. This experiment is all the more convincing because it was performed on healthy young men. In its simplest form it consists in crowding as many persons as possible into an air-tight cabinet, provided with an electric fan, and with the necessary apparatus for measurements of the physical and chemical condition of the air. In describing the results of this experiment, I can not do better than quote from Leonard Hill, who, though not the first to perform the experiment, has so greatly extended our knowledge of the science of ventilation during recent years.

"After 44 minutes the dry-bulb thermometer stood at 87° F., the wet bulb at 83° F. The carbon dioxide had risen to 5.26 per cent. The oxygen had fallen to 15.1 per cent. The discomfort felt was great; all were wet with sweat and the skin of all was flushed. The talking and laughing of the occupants had gradually become less and then ceased. On putting on the electric fans and whirling the air in the chamber the relief was immediate and very great, and this in spite of the temperature of the chamber continuing to rise. On putting off the fans the discomfort returned. The occupants cried out for the fans. No headache or after-effects have followed this type of experiment which has been repeated five times." Long before the discomfort had become extreme the oxygen percentage became so low that matches would not light. The disinclination to smoke cigarettes was not noticed until some time after it was impossible to light them.

In other experiments of similar type the person in the cabinet was allowed to breathe outside air through a tube, but with no amelioration of the uncomfortable feeling, or a person outside the chamber breathed for hours the air inside it through a tube without suffering any discomfort. Clearly therefore neither the chemical nature of the air, nor the presence of toxic substances in it, has any relationship to its evil influence. But the experiment is not merely destructive of previously held hypotheses; it also points the way to the true solution of the problem, for it indicates that stagnation of air loaded with moisture has some very close relationship to the discomfort. It shows that a change in the *physical* rather than the chemical properties of the air is the real cause of its deleterious action.

These changes can affect but one function of the body, namely, that of heat dissipation, and by so doing cause disturbances in the mechanism of heat control. This does not necessarily imply that this disturbance is so great as actually to cause an increase in the body temperature, although this is very commonly observed in persons who have been for some time in crowded places, but it interferes with a mechanism which is responsible not alone for proper heat regulation but also for the maintenance of a correct relationship of blood supply to different parts of the body, and for tonic stimulation of the nervous system.[3]

The mechanism by which the body temperature is maintained is in part like that of a radiator, the temperature of which depends, first on the rate at which the furnace is burning and second on the cooling influence of the air in contact with the radiator. The physical properties of the air which determine the cooling are those which influence radiation, conduction and convection. In the animal body, these processes come into play mainly at the surface of the skin, where, however, excessive loss is guarded against partly by the low conductivity for heat of the skin and of the subcutaneous tissue (fat), and partly by the fact that the blood supply to the skin is scanty compared with that of the deeper tissues, so that the blood flowing in the skin has a decidedly lower temperature than that in the tissues a few millimeters deeper. This relationship of superficial and deep temperatures is maintained by the action of vasomotor nerves to the blood vessels, and whenever the body is exposed to warmer air, the vessels of the skin become dilated so as to draft more blood from the deeper to the superficial vessels, causing flushing of the skin. Flushing of the skin is therefore a normal reaction, but at the same time it is a warning that the heat-regulating mechanism is being put on a strain.

But these mechanisms are inadequate to account for all the heat loss, for man can withstand temperatures that are not greatly below those of his body, indeed he can tolerate for some minutes temperatures that are higher. It is recorded, for example, that two observers exposed themselves for a short time in an oven in which a steak was cooking, and it is well known that certain miners work for considerable periods at very high temperatures.

Evidently some other mechanism independent of the cooling effect of air itself, and not acting in the case of a radiator, comes into play. This is evaporation, and it occurs at two places in the body, at the surface of the skin, where sweat is evaporated, and in the lungs where the expired air is saturated with water vapor. The physical factors which control the degree of heat loss by evaporation at these two places, are not precisely the same. In the case of the lungs the inspired air becomes saturated with water at body temperature, and the amount of evaporation necessary to do so depends upon the amount of water already contained in the inspired air, that is, on the *absolute* humidity; the lower this is, the more water will it require to effect saturation.

In the case of the evaporation of sweat, the amount of moisture vaporized from the body depends on the temperature and the *relative* humidity of the atmosphere.

It is in connection with this phase of the subject, more than any other, that many people find it difficult to understand the true significance of relative humidity to the well-being of the body. The difficulty depends on the fact that the relative humidity has an opposite influence at low and high temperatures. In the former case it increases the conductivity of the atmosphere for heat and has a cooling influence, and in the latter it interferes with the evaporation of sweat, and has a heating influence. Below about 65° F. the cooling effect of moist air is prominent because there is little sweating, therefore a cold wet atmosphere is chilling—it conducts heat away. At about 70° F. the cooling effect of air disappears and sweating occurs. The evaporation of the sweat now causes cooling, the degree of which varies inversely with the relative humidity. Between these

two temperatures, i. e., 65° and 70° there is a range in which humidity has little influence—a neutral region. The influence of high relative humidity on bodily comfort at temperatures above the neutral temperature becomes very marked indeed; at 85° F. and a relative humidity of 90 per cent, for example, very serious symptoms appear in a few minutes, when there is no movement of the air.

Relative humidity and temperature alone are not, however, the only physical conditions to be considered. Another is *the movement of the air,* for even under the unfavorable conditions just cited, immediate relief is afforded if an electric fan be started, as it will be recalled was the result in Hill's experiment. The movement of the air enables it, though nearly loaded to its full capacity with moisture, to carry away considerable quantities in small loads.

The wearing of clothes greatly affects the rate with which these changes occur. The clothes act as barriers, preventing the movement and exchange of air around the body. The garment next the skin entraps a layer of air which is more or less at the same temperature as the skin, and which soon becomes saturated with moisture at that temperature. Between the inner garments and those over them other layers of air are entrapped, each one being at a somewhat lower temperature and containing less moisture than the one inside. These layers of air, therefore, form stepping stones, as it were, between the extreme conditions next the surface of the skin, and the environment of the clothed body. Obviously if the layers of air next the skin are to be renewed at such a rate that they remain cooler than the skin and unsaturated with moisture, the clothing must be adjusted to suit the outside conditions.

There is every reason for believing that it is because of interference with these processes that improperly ventilated and overcrowded places are uncomfortable. The moisture exhaled and evaporated from the bodies soon raises the relative humidity so that heat loss is retarded from the skin, and the heat that is actually given off raises the temperature so that loss from the body by radiation and convection becomes suppressed. As the temperature steadily rises, the air takes up more and more moisture, with the result that less and less heat comes to be lost from the lungs in saturating the expired air with vapor. The physical conditions of the environment become unsuitable for the physiologic mechanism of heat loss, although meanwhile heat production goes steadily on. The body furnaces are not damped down in proportion as the loss of heat diminishes, and the consequence is a rise in the temperature of the blood—a mild fever. Now it is well known that the cellular activities, which, taken together, make up the life process of the body are extraordinarily sensitive to change of temperature; their chemical activities become interfered with, they demand more oxygen, they fail to get rid of effete products properly, substances which have no action on them under the ordinary conditions of temperature become toxic and so forth. A highly abnormal internal environment therefore becomes created around the living tissues of the body.

But short of a measurable rise in the temperature, improperly ventilated places cause reactions in the human body that are responsible not only for the discomfort which is experienced, but also for a lowering of resistance to infections. These reactions are due in the first instance to alteration in the temperature differences between the skin and the underlying tissues. Normally, as has

been remarked before, this difference maintains at the skin a constant stimulation of the thermic nerves, and this stimulation is important in maintaining the tone of the nerve centers. The nerve cells that control the functions of the body do not originate impulses; they only act when other, afferent impulses arrive at them. There are many varieties of stimuli which may excite these afferent impulses, but none more important than those which excite the heat nerves of the skin. This stimulation depends on the rate at which heat is passing through the sense organs (or receptors), in which these nerves terminate. It is necessary to emphasize that it is the rate of change that acts as the stimulus, and this depends on the difference between the deep and superficial temperatures. When the skin vessels become dilated so large a volume of blood reaches the surface that this difference becomes slight, and the thermic receptors are not stimulated. There are many practical applications of these principles; thus it is because of stimulation of the thermic skin nerves that cold baths have a bracing effect, that the open-air treatment, as in tuberculosis, tones up the body and enables it the better to hold its own against the tubercle bacillus, and that sleeping out of doors is the best tonic for maintaining good health. In open-air treatment it is true that the body is closely wrapped up—that is essential—but this does not eliminate the cooling influence, for not only does the cool air play on the exposed face and hands, in the skin of both of which the thermic nerves are very sensitive, but it acts also on these nerves in the skin, under the clothes, for the clothing merely serves to regulate the rate of cooling. This still goes on very much more than it would with much less clothing in an atmosphere that is stagnant, hot, and humid. Open windows in bedrooms are never so healthy as open-air porches, because there is no draft. It is the draft that is important. Naturally it must be regulated so that it is not restricted to one part of the body only—that obviously would introduce conditions to which the body is unaccustomed—it must blow equally all over. There is probably no greater fallacy in popular hygiene than that drafts are dangerous. Like all good and desirable things they become so only when they are improperly used—when a person overheated by being in a hot atmosphere is suddenly subjected to a restricted draft, of course there is danger that the sudden change of conditions, affecting one part of the body only, will cause vascular disturbances that may be undesirable, but if the conditions be properly controlled, drafts are the healthiest stimulants and the best tonics.

This brings us to a problem in ventilation that is attracting very considerable attention at the present time, namely, the relationship between ventilation and infections. It is a common experience not only that ordinary colds, but more serious infections as well, can be directly traced to some unsuitable condition of ventilation; such as sudden exposure to a draft while overheated, or going out into a cold, damp atmosphere from an overheated room. What is the reason for the infection under these conditions? At the outset we must recognize that all these conditions, colds, catarrhs, bronchitis, just like the more acute infectious diseases like diphtheria, pneumonia, cerebrospinal fever, etc., are due to microorganisms, and the question therefore is, why should unfavorable ventilating conditions so frequently be the immediate cause of the attack.

There are two methods by which the infection might occur. First, by a great increase in the number of organisms in the air, and secondly by a lowering

of the resistance of the body towards the organisms, which would not then require to become increased in numbers. The former method is usually known as mass infection, and there can be no doubt that it is very common, perhaps, indeed, is the commonest cause for infection. The organisms, of course, come from infected individuals, who add them to the atmosphere in the exhaled air, particularly when this is forcibly discharged as in coughing or sneezing, or even in speaking.

I need recall only a few observations in order to illustrate the importance of this factor. If the mouth be rinsed with a culture of some readily recognizable organism not commonly present in detectable amounts in the atmosphere, and the person, standing in front of a row of Petri dishes, each containing some culture medium upon which the organism will grow, then speaks at ordinary pitch, the plates, after proper incubation, develop colonies of the organism, those nearest the speaker having most, but even those at a distance of several feet also showing them.

A serious problem in zoological gardens has been to keep animals that are highly susceptible to tuberculosis free from this disease. The higher apes, for example, inevitably succumb to it, being infected by the tubercle bacilli exhaled by persons standing in front of their cages, many of whom harbor these bacilli, though they may not show any of the symptoms of tuberculosis. Now it has been found that if glass screens are erected in front of the cages, the animals remain almost free from the disease.

But mass infection does not suffice to explain the cause for the onset of attacks of many conditions that are, nevertheless, fundamentally due to bacteria, such as ordinary colds. These can frequently be traced to some chill, or wet feet, or exposure to sudden change in temperature. In such cases it is believed that the bacteria are present on the mucous membranes of the upper respiratory passages, but that they remain inactive because of the normal protective influences which exist on these surfaces. So long as the blood supply is normal, these protective influences are adequate to protect the body from invasion, but if this should become curtailed, then the bacteria become active and set up pathologic processes. Evidence favoring this view has been obtained by several recent investigators by finding that the blood supply of the upper respiratory passages becomes decidedly curtailed when the surface of the body is cooled. For example Leonard Hill and Muecke[1] some years ago examined with a speculum the mucous membranes of the nose under various conditions, particularly out of doors, and in rooms which were ventilated and heated to an average degree. Out of doors the mucosa was pale and taut, and when touched by a probe did not show any pitting. This is the normal condition. Indoors it was common to find the membrane decidedly swollen, flushed with blood and covered with thick secretion, and when a probe was pressed on it a depression resulted lasting for some time. In one case that was frequently examined during these observations there was a deflected septum which only partly blocked the nasal passage on one side when the person was outside, but which did so completely under unfavorable conditions of ventilation. It is this swelling of the nasal mucosa and probably of that of the cavities which extend upward from it on to the forehead that causes the sense of stuffiness and probably also the headaches which are common in crowded, overheated places.

The conditions found to bring about these changes with greatest certainty were when the feet were cold and the air round the head was warm, conditions which are just exactly the opposite of those obtaining out of doors. Here the head is usually more quickly cooled than the feet because convection currents of cool air play around it freely, whereas next the ground the air is more stagnant. Besides, if the sun is shining the earth becomes heated by absorbing the heat. The temperature as registered by a thermometer, either wet or dry bulb, may be the same at the feet as at the head. It is not this that counts, however, it is the rate of cooling which is dependent, mainly, on the movement of the air. Now in a poorly ventilated room, such for example as one heated by a stove, or even by radiators, and in which there is no movement of air, the feet become cooler than the head, and it is under these conditions that the nasal membranes become swollen. It ought to be emphasized that the cause for these changes is not cold feet alone. It is the combination of cold feet and hot head. Out of doors, it is well known,, that any one may stand with cold feet for hours without any risk of catching cold, but then the head is really cooling as fast as the feet, because of convection currents.

The ideal system of warming a room is to supply radiant heat near the floor level; open fires, properly flued modern gas fires, and electric heaters at floor level are the best methods to attain this.

Suppose now the person subjected to conditions which cause the mucous membrane to become swollen and congested should go outside, then the membrane at once becomes pale because the blood vessels constrict, but for some time it remains swollen and boggy and continues to show pitting with a probe. It is while in this state that it offers favorable conditions for the growth of bacteria. The membrane is swollen and covered with secretion, and the blood flow is cut down. The natural defensive agencies that are normally carried by the blood do not succeed in combating the multiplication of the bacteria in the swollen membrane. After some time out of doors the blood supply returns because it is required to warm up the cool air, but this reaction does not occur before the mucosa has regained its normal condition.*

The protective influence of a rapid blood flow through the nasal membrane is possibly the explanation of the relative immunity from infectious colds of those who work in air containing irritating gases, such as workers in various kinds of chemical factories. Even the irritation set up by coal dust may, by similar methods, afford some protection against infection by the tubercle bacillus,—for phthisis is relatively infrequent among coal miners. The supposedly antiseptic action of ozone is probably due to a similar irritating effect. Any benefit that may be derived from its presence in the atmosphere can not otherwise be explained. It is possible that a useful prophylactic practice to avoid infection, such as that of influenza, would be to stimulate the nasal mucosa at intervals by snuff, but this may be an unwise suggestion.

After becoming acclimatized to outdoor conditions, the nasal mucous membrane is in a much more favorable condition to withstand infection than indoors because of the very rapid blood flow that is necessary in order to supply heat

*The congestion of the mucous membrane brought about by warm moist air does not probably depend on dilatation of the small arteries—entailing increased flow of blood—but rather on dilatation of the capillaries and therefore a stagnation of blood.

with which to warm up the inspired air. This more rapid blood flow, and the freer flow of lymph which accompanies it, is reinforced by increased secretion, which assists to wash away invading bacteria. Mass infection being equal inside and outside, the animal body can withstand it much less satisfactorily in the former case.

These observations on the reactions of the respiratory membranes to atmospheric conditions have been confirmed by other investigators. Thus Cook[4] caused persons to breathe forcibly through the nostrils onto a mirror surface, and then marked on it with a wax pencil the outlines of the moisture deposited. Although the extent of the outlines varied somewhat with the depth of breathing, they afford a general estimate of the width of the air passages. It was found that there is marked reduction in the nasal air passage in a warm room. Winslow[5] also sums up the observations (150 in number) made on this aspect of the problem, in the following words: "Ordinarily it was found that heat causes a swelling of the inferior turbinate of the nose, tending to diminish the size of the breathing space, increased secretion and reddening of the membranes. The action of cold is, as a rule, just the opposite."

Many other observations bearing on the relationship between chilling and immunity to infection have been recorded, but it would take us beyond our subject to discuss them here. Because of their accuracy and the excellent control of possible fallacies, it is important, however, to say something about the recent investigations of Mudd and Grant.[6] These observers measured the temperature of the mucous membranes of the palate, tonsils, and pharynx by means of thermocouples before and during application to the skin of cold towels, or while cold air from a fan was allowed to play on it. A rise in temperature would indicate that the part had become more vascular, and a fall, the contrary. That this interpretation was the correct one was confirmed by direct inspection of the degree of flushing (redness). It was found that chilling the body surface immediately caused a fall in the temperature of the mucous membranes which could not be accounted for by any accompanying change in blood pressure, or, entirely at least, by changes in respiration or by lowering of the temperature of the blood. The conclusions are "that chilling of the body surface causes reflex vasoconstriction and ischemia in the mucous membranes of the palate, faucial tonsils, oropharynx and nasopharynx."

And now the final question presents itself. What are the ideal conditions of ventilation? It is a most difficult question to answer, and one over which at present several large commissions are at work. Indeed most elaborate experiments have been planned and undertaken to throw light on the question. The observations of the New York Commission on Ventilation, and those conducted by Mr. Watt, in the Graham School in Chicago, are among the most important in this country, and, of course, they interest us much more directly than those conducted on the other side, where the climatic conditions are fundamentally different.

Many of the observations have so far been made on properly selected groups of school children, taught in classrooms with different ventilating conditions. Attention is directed to the general efficiency of the pupils and the condition of their health. The temperature, and the humidity of the air are the physical conditions of the atmosphere which have been more particularly studied, but a

great deal more work must be done before any definite conclusions can be offered. It appears, however, that for school room air a temperature of 65-68° F., with a relative humidity of 45-60°/° is the optimum. To maintain these conditions throughout the period a class occupies the room, usually requires, in this country, at least, the addition of a considerable quantity of moisture to the ventilating air. The air of most of our school rooms in winter errs on the side of being too dry, for under these conditions the mucous membranes suffer injuriously. An excellent summary of the various authoritative conclusions with regard to the optimum conditions of ventilation for class rooms is given by Burnham in the Pedagogical Seminary.[7]

Although the present review does not venture to discuss the methods that are employed for the measurement of the various physical properties which have to be considered in gauging its influence on health, or the engineering problem of how ideal conditions may be maintained, it may not be out of place to mention, in connection with the former of these, that the physical property to which most attention should be devoted is the cooling power. This can not be done by reading an ordinary thermometer, for this instrument only registers the temperature of the piece of wood and of the wall against which it is hung. It registers the same whether the air is dry or moist, or whether it is stagnant or moving. Somewhat more information regarding cooling power is afforded by readings of a wet-bulb thermometer, an instrument in which the bulb is kept constantly moist, so that evaporation occurs from it. This evaporation tends to cool the thermometer, in proportion to its rate, and since this is dependent mainly on the degree to which the air can take up more moisture, we can tell by the use of a formula or tables the relative degree of humidity of the air. Still this does not tell us the real degree of cooling which the atmosphere can bring about. It does not adequately register the cooling which is dependent upon movement in the air, the so-called convection currents. To afford this information, Leonard Hill has invented what he calls the Kata thermometer, by which the rate of cooling is directly measured. The instrument consists of an alcohol thermometer with a relatively large bulb, and with the scale registering between 105° F. and 90° F. It is placed in warm water at about the former temperature, and is then removed, and the time required for the temperature to fall from 100° F. to 95° F. is measured by means of a stop watch. This time divided by a factor determined for each instrument, and written on the stem, gives the actual amount of heat in millicalories per square centimeter per second which would be given off from, say the surface of the human body, under similar environmental conditions. Hill and his associates[3] have shown that much important information concerning the cooling power of the atmosphere can be gained in this way, which can not be gained by any other.

REFERENCES

[1]Hill, Leonard, and Muecke, F. F.: Lancet, London, 1913.
[2]Hill, Leonard: Jour. Physiol., 1910, xli, 3.
[3]Hill, Leonard: The Science of Ventilation and Open Air Treatment, Medical Research Committee, H. M. Stationery Office, London, 1919.
[4]Cock, H. Girard: Trans. Am. Laryngol., Rhinol., and Otolog. Soc., June, 1915.
[5]Winslow, C. E. A.: Am. Jour. Pub. Health, 1917, No. 3, p. 157.
[6]Mudd, S., and Grant, S. B.: Jour. Med. Research, 1919, xl, p. 53.
[7]Burnham, W. H.: The Pedagogical Seminary, 1919, xxvi, 311.

—*J. J. R. M.*

Influenza

WITH the increase of influenza once more to epidemic proportions it may be of advantage to review some of the work that has been reported since the epidemic of 1918, concerning the clinical phenomena of the disease. Given a case of illness with sudden onset, prostration, headache and general body pains, with febrile period of fixed duration, and without complications or other causative pathology, such as acute tonsillitis, acute coryza, trichiniasis, accessory sinus disease, etc., and in the absence of an epidemic, one can say that the condition is apparently influenza, but there is no method by which one may prove that it is the same disease as that which caused the pandemic of 1918. There is no one diagnostic sign or group of signs such as we possess in the exanthemata, by which the diagnosis may be finally concluded. It might be helpful to include in the description a statement of its extreme contagiousness and its epidemic propensities. When contagion can be demonstrated it is surely of help in making a clear-cut diagnosis. But there is abundant evidence that in interepidemic cases the contagiousness is by no means as great as during the explosive outbreaks.

Nevertheless, epidemic influenza is a clinical entity caused by a specific organism, and in all probability the interepidemic cases and surely those postepidemic cases which have occurred since the winter of 1919 are due to the same cause. Whether that cause be B. influenzæ or some other unknown virus is not a matter for discussion in this paper.

What is influenza? This question is no easier to answer than is the equally complicated one propounded by Rivers, "What is an influenza bacillus?" It is a disease with certain clinical manifestations as indicated above. Unfortunately similar manifestations may accompany the onset of other infections not influenza. Acute tonsillitis may produce fever, prostration, headache and general body aches. Again, sore throat may be present in influenza, although as a rule, this is mild and the tonsils do not show exudation, ulceration or the intense local inflammation of an acute follicular tonsillitis. Acute coryza is easily distinguished. The absence of systemic symptoms, the more profuse and irritating nasal discharge and the subsequent development of tenacious purulent secretion serve to distinguish it from influenza. In the course of the disease we may through negative findings rule out the other specific infectious diseases. Perhaps the greatest difficulty lies in distinguishing between the mild interepidemic variety and cases of tonsillitis and pharyngitis with constitutional symptoms but without great visible local pathology. Of help here is the delayed return of strength and tonus, the postinfluenzal asthenia which follows even mild infection with the true disease, although even this symptom is less pronounced than during epidemics. In influenza there is no swelling of the lymph glands of the neck as is so often the case in tonsillitis. The presence of leucopenia is a corroborative finding of great value. It has further been found that in the postepidemic cases of 1919 leucopenia has remained characteristic of the disease.[1]

A dusky erythema of the skin, particularly over the face and neck, has been described by Bloomfield and Harrop,[2] an erythema different from the simple flush of fever and not dependent on cyanosis; one which persisted into convales-

cence and was often followed by desquamation. It is likened in character to that accompanying scarlet fever or urticaria, or following burns. They also describe intense hyperemia of the mucous membrane of the buccal cavity somewhat similar to that seen after the application of a chemical irritant such as adrenalin, and which usually subsided after two or three days. The skin erythema has been described by many writers.[3, 4, 5, 6] The face appears hot and flushed. This, with the conjunctival injection and slight coryza, resembles the appearance of an early measles patient in whom the rash is not yet distinct. At times the influenza rash may be punctate in character. Bloomfield reports that among the postepidemic cases studied the skin and mucous membrane lesions have been present in only slight degree. In them the mucous membrane hyperemia is indicated only by a slight diffuse reddening of the pharynx, pillars and soft palate, and is accompanied by swelling of the lymphoid tissue of the pharyngeal wall.

Hemorrhagic tendency is indicated by epistaxis, hemoptysis, precocious menstruation, profuse bleeding in abortion, and more rarely, hematemesis and purpura.

Arrived at a consideration of those cases which have developed pulmonary symptoms—bronchitis or pneumonia—we are again confronted with an absence of pathognomonic physical signs. One of the most constant and one which aids greatly in establishing the diagnosis, is the unusual degree of cyanosis which is most easily recognized on the mucous membrane of the lips and on the cheeks and ears. This sign comes early, in fact is considered by some to accompany the uncomplicated influenza. We do not know of any work that has been done to determine how early in the disease it occurs. Harrop[7] uses the term cyanosis synonymously with the "dusky erythema" previously referred to. The majority of observers speak of the cyanosis as a manifestation of complicating pulmonary involvement.

The cyanosis exists to an extent out of all proportion to the amount of dyspnea present. In fact in the early pneumonia there is relatively little increase in respiratory rate. The cause of cyanosis has received considerable study. Torrey and Grosh[8] report that a large proportion of their series of cases during the epidemic exhibited clinical signs of pulmonary emphysema. There was an apparent increase in the circumference of the chest during the illness, shallow respiratory excursion, tympany under the sternum, vertebral column and much of the precordium, low standing diaphragm and often bulging supraclavicular fossæ. At autopsy the lungs showed well-formed rib markings and emphysematous bullæ under the visceral pleura. The lungs could not be collapsed by pressure, and even the apparently solid portions floated high in water. The lung tissue was usually fairly tough at the apices, but extremely friable at the bases where it was easily disintegrated under moderate pressure. Torrey and Grosh conceive of damage to the parenchyma, done perhaps by interference with the normal blood supply by the nodules of peribronchial infiltration, and subsequent rupture of the damaged alveolar walls. Their explanation of the cyanosis, as well as the dyspnea and the epistaxis, is in the pulmonary emphysema which, through increasing the intrathoracic pressure, caused some degree of venous stasis. In support of their theory they state that in those rather frequent cases

which developed interstitial emphysema, there was an immediate amelioration of symptoms, with lessened respiratory distress, diminished signs of thoracic inflation, and some lessening of the cyanosis. The fall in intrathoracic pressure following passage of the air into the tissues of the mediastinum lessened, in their opinion, the venous stasis.

Whether or not we accept this explanation for the cyanosis, two facts mentioned by these authors have been established in the experience of many observers during the last epidemic. The pathologists in particular have had opportunity to observe the damaged alveolar walls[9] with frequent emphysematous patches, and all clinicians who have treated any large number of cases can report instances of interstitial emphysema in which the air often passed not only into the upper and lower mediastinum, but up through the fascial tissues of the neck. In extreme cases the emphysema involved the subcutaneous tissue from the region of the eyes downward to the groin. A case reported by Clark and Synnott[10] is of interest in that the discovery of gas in the mediastinal tissues at autopsy explained some very unusual sounds heard through the stethoscope: "over the precordium rales very similar to sounds elicited with moderate pressure of stethoscope over emphysematous cutaneous tissue" were heard.

Kelman[11] observed the frequency of vesicular emphysema which, in her experience, was usually marginal with not infrequent emphysematous bullæ discovered substernally. The roots of the lungs were also quite commonly involved. She also observed the dyspnea and cyanosis which were out of proportion to the amount of lung involvement. Experimenting on rabbits, with artificial pressure inflation of the lungs, she reached the following conclusions: "Increased intrapulmonary pressure causes rupture of some of the superficial distended air vesicles and there is an escape of air underneath the visceral pleura, which is rather firm. The air finds less resistance in escaping underneath the pleura toward the hilus than to break through the pleura. From the hilus it travels along the pleural reflexions over the pulmonary arteries and veins to where it meets the pericardial reflexions over the same vessels, the tissues of the mediastinum, especially the pericardium and pericardial fat, thence it extends to the superior mediastinum and passes downward into the retroperitoneal tissues—the perirenal tissues being most commonly invaded." It may pass on down by the great vessels into the thigh, or superiorly, following the subclavian into the axillary space. That she did not produce cervical emphysema is explained in part by the fact that the neck tissues of the rabbit had been opened for tracheotomy. Kelman showed that in death from anaphylaxis both vesicular and interstitial emphysema may be present. She also showed in living rabbits that emphysema itself was capable of producing dyspnea and cyanosis. She conceives of the vesicular emphysema of influenza as resulting from the strain of coughing on lung tissue damaged by the influenza toxin.

Hoover[12] studied cyanosis and dyspnea in gassed soldiers. The probable cause as described by him might play some part in the cyanosis of influenza pneumonia. Foam in the exudate of the alveoli interferes with the normal transfer of oxygen and carbon dioxide between the alveolar air and the blood. Any portion of the respiratory membrane which is covered by alveolar air foam is deprived of its normal respiratory function, both for the escape of carbon diox-

ide and for the absorption of oxygen. If a lobe of the lung is filled with this foam, the surface area for gaseous interchange is no longer the very large area of the respiratory epithelium of the lobe, but the cross section of the bronchus leading to the lobe,—the surface area of the foam in the bronchus. Hoover showed that gassed soldiers, whose lungs contained much alveolar foam, when breathing pure oxygen, recovered within a few minutes from their cyanosis but obtained no relief from the associated dyspnea. Within these few minutes the pure oxygen had been well mixed with and absorbed in the alveolar foam, thence gaining access to the blood and relieving the cyanosis. But the concentration of carbon dioxide in the foam remained the same as when atmospheric air was being breathed and the amount in the blood remained unchanged, so the dyspnea persisted. Hoover's work was reported before the epidemic of 1918 but a case described by him may be quoted as an example of the type. "A young man who had bronchitis with an abundance of moisture in his air spaces, had pronounced cyanosis of the lips and fingers. There was only a moderate dyspnea. It did not amount to air hunger. There were no demonstrable areas of consolidation in the lung. * * * He breathed oxygen through the closed system and after he had inhaled it for about three minutes, all traces of cyanosis disappeared. But he breathed with exactly the same rate and volume as when he was breathing atmospheric air."

The oxygen and carbon dioxide content of expired air corresponds to blood content of these gases only so long as the entire alveolar respiratory membrane is functioning properly. When moisture covers portions of the alveoli the gaseous tension of the alveolar air no longer corresponds necessarily to that of the blood, and furthermore the variation may be unequal in the case of the two gases.

That the cyanosis of influenza pneumonia is mechanical rather than biochemical is indicated by the work of Harrop[7] who found that both the oxygen content and oxygen unsaturation of the venous blood showed normal values in both uncomplicated influenza and influenza pneumonia, and particularly by the work of Stadie[13] who confirmed these results and further showed that an increase of cyanosis was accompanied by an increase of unsaturation of the arterial blood. The cyanosis is then due to incomplete saturation of venous blood with oxygen in the lungs. Neither Stadie nor Harrop found any decrease in the oxygen carrying capacity. Methemoglobin formation, if it did occur at all, could not have been present to such an extent as to have been a factor in producing the cyanosis.

A discussion of the clinical phenomena of the disease would scarcely be complete without brief mention of the characteristic lung picture at necropsy. Here at last we may find signs that promise to give as reliable evidence as do Koplik's spots in measles, or rose spots in typhoid fever. The particular lesion has been described by Wolbach,[9] Goodpasture and Burnett[14] and by MacCallum[15] and consists in dilation of the alveolar ducts with a hyaline membrane partially or completely covering their walls and sometimes those of subtended alveoli. These are the pathologic reactions which have been found in no other disease, but other pathologic changes accompany these and are found elsewhere in the

lung substance. The other changes are similar in character to those described by MacCallum in post measles pneumonia.

In conclusion we may state that influenza is a disease of unknown or undemonstrated etiology which bears many clinical characteristics analogous to those of measles and kindred exanthemata.

REFERENCES

[1]Bloomfield, A. L.: Medical Clinics of North America, 1919, p. 1635.
[2]Bloomfield, A. L., and Harrop, G. A.: Bull. Johns Hopkins Hosp., 1919, xxxi.
[3]Frankel, B.: New York Med. Jour., 1918.
[4]Conner, Lewis A.: Jour. Am. Med. Assn., lxxiii, 321.
[5]Locke, E. A.: Rönne, G. E., and Lande, H.: Boston Med. and Surg. Jour., 1919, cl, 124.
[6]Cowie, D. M., and Beaven, P. W.: Jour. Mich. State Med. Soc., 1919.
[7]Harrop, Geo. A., Jr.: Bull. Johns Hopkins Hosp., 1919, xxx, 10.
[8]Torrey, R. G., and Grosh, L. C.: Am. Jour. Med. Sc., 1919, clvii, 170.
[9]Wolbach, S. B.: Bull. Johns Hopkins Hosp., 1919, xxx, 104.
[10]Clark, Elbert, and Synnott, Martin J.: Am. Jour. Med. Sc., 1919, clvii, 219.
[11]Kelman, Saral R.: Arch. Int. Med., 1919, xxiv, 332.
[12]Hoover, C. F.: Jour. Am. Med. Assn., 1918, lxxi, 880.
[13]Stadie, W. C.: Jour. Exper. Med., 1919, xxx, 215.
[14]Goodpasture, E. W., and Burnett, F. L.: U. S. Naval Med. Bull., xiii, No. 2, p. 177.
[15]MacCallum, W. G.: Jour. Am. Med. Assn., 1919, lxxii, 720.

—*W. T. V.*

A Medical Man's Opinion Concerning Universal Military Training

I AM often asked whether I approve of Universal Military Training. Under certain conditions I do; under other conditions I do not.

If we are to have Universal Military Training and our sons are to be mobilized and sent to camps in the way that was done in 1917, I am unalterably opposed to Universal Military Training. Under these conditions I would feel that it would be criminal to advise those who have confidence in me to permit their sons to be mobilized and encamped as they were in 1917. At that time it was pointed out to the authorities that the method of mobilization then followed would gather into the camps all infections existing in the areas from which the soldiers came. The drafted men were brought together in villages and cities, they came clean and unclean, bearing the bacteria of all the homes and localities from which they came. In their ordinary clothing they were huddled together until a certain number accumulated, when they were placed on troop-trains and sent to the designated camp. In the fall of 1917 not a troop train reached Camp Wheeler without having on board from one to six cases of fully developed measles.

The men on this train had been en route for from twelve to forty-eight hours. Practically every man on every train, certainly every man in certain cars, had been exposed to measles. To control the disease under these conditions is beyond the power of the most skillful epidemiologist.

In the spring of 1918, several hundred drafted colored men were assembled at Montgomery, Alabama. As in other instances they came in their ordinary clothing, more or less soiled, generally more, bringing the bacterial flora of hundreds of cabins in the State. They were held together until the desired number assembled, then they were placed on troop trains and after a journey of from two to three days reached Camp Custer. Some sixty cases of streptococcus

pneumonia were taken directly from the train to the base hospital, and within a month the type of pneumonia throughout the whole division at Camp Custer was changed. Instances of this kind might be multiplied many times. What was the result? This can be ascertained by comparing the death rates from the different infectious diseases in the camps with the death rates in those who remained at home, belonging to the same age group. When we make this comparison, what do we find? The death rate from pneumonia per 100,000 was 9 times greater in the army; from meningitis, 15 times greater; from scarlet fever, 2.5 times greater; from diphtheria 1.1 times greater; from measles 6 times greater; from all causes 1.2 times greater. It must be remembered that tuberculosis was largely excluded. The mortality from this disease was 8 times greater in the civilians of that age group.

If Universal Military Training is to be adopted, the health of those mobilized and called to camp must be so guarded that the death rate must not be higher than among those of the same age group at home. Can this be done? I think that it can, but if it is done, the examination of men to be sent to the camps, their mobilization in their home neighborhood, their transportation, their care after they reached camp must be under the control of our best epidemiologists.

In 1917, army officers stated with truth that the purpose of mobilization is not to make a demonstration in preventive medicine, but to assemble civilians, and to convert them into efficient soldiers as quickly as possible. This explanation was valid at the time, and under the then existing conditions; it would not be valid in peace times. Our unpreparedness in 1917, and the hurried mobilization of troops and their intensive training were absolutely necessary. It was suggested at that time that more sanitary methods of mobilization and handling the drafted men might be put into practice. If we are to have Universal Military Training, the young men of the designated age should be assembled in groups of not more than 30, carefully examined, stripped of their ordinary clothing, barbered, bathed, and put in sterilized uniform, then they should be held in quarantine in some armory hall or in tents or possibly they might be under certain conditions billeted. They should be held for at least 14 days with daily examinations, of course with the exclusion of any infection that might occur during this time. During this period they should receive their vaccinations and at the end of this period each group should be placed in a car, not a troop train, or at least each group should be isolated and sent to the camp. After reaching the camp the isolation of each group should be continued during 14 days longer. This would not mean that they could not be drilled and put through proper exercise during this time. All drilling might be done with the groups while detached; in addition to this the camps must be in perfect sanitary condition. There is no difficulty in securing this. Our camps in 1917 and 1918 were in better sanitary condition by far than the average village or city in the United States. It may be asked whether one has a right to assume that under conditions as just specified our young men could be mobilized without increased death rate. In our larger universities from five to twelve thousand young men and women from all parts of the world assemble every fall without an increased death rate. In my opinion this renders it not only highly probable but quite certain that under proper conditions, mobilization of the young could be practiced without increased morbidity or mortality.

The S. A. T. C. had in it the germ of great possibility of good. It was hurriedly and imperfectly conceived and put into being. The epidemic of influenza came and wrought havoc. The war ended and the people in general are sick of everything military. We will probably continue in this attitude until we are forced to mobilize hurriedly as we did in 1917 with the same great loss of life. I certainly believe that physical training with physical, not military drill, should be introduced into all of our high schools. Every young person attending these schools, girls as well as boys, should undergo a careful physical examination, not a perfunctory one, at least once a year, better twice a year, and all should be drilled, not military drill, but in the most approved forms of physical training. It is to be hoped that our school authorities will appreciate the value of physical training. I would be opposed to military drill in our high schools. Military maneuvers do not furnish the best forms for the physical education of the young. The periodical examinations should enable us to group our young men and women and point out to each what manner of life is best suited to his or her physical condition. Hundreds with incipient tuberculosis go into occupations and professions which hasten the development of this disease. If it had been recognized in its earliest incipiency, the individual might have been guided and instructed in some other vocation, which would have been more suitable to his physical condition. In the past we have waited until the young man has established himself in some trade or profession when he shows signs of developing tuberculosis and we say, he must go and live an outdoor life. The lawyer, the doctor, the manufacturer, the carpenter, after they have established themselves in a special line of work must become a gardener or a farmer. He soon finds that as gardener or farmer he is not a success. He can work successfully only in the trade or profession which he has learned. He goes back to the shop, the office, the counting room, spreads tuberculosis among his fellows, and dies from the disease himself.

I certainly believe in physical preparedness of the race, but attempts to provide for it must be scientific and not haphazard.

—*V. C. V.*

Raise In Subscription Price

With the February issue of the Journal of Laboratory and Clinical Medicine, the subscription price will be raised to $5.00. As a reason for this increase, we only need to point to the fact that cost of printing and paper, and the making of illustrations, has increased from one hundred to three hundred per cent since the Journal was started four years ago.

The publishers feel that the readers of this Journal prefer to pay an additional cost of two dollars a year rather than have the quality of the Journal lowered in any particular.

Government Needs Physicians

The United States Civil Service Commission announces that a large number of physicians are needed for employment in the Indian Service, the Public Health Service, the Coast and Geodetic Survey, and the Panama Canal Service.

Both men and women will be admitted to examinations, but appointing officers have the legal right to specify the sex desired when requesting the certification of eligibles.

Entrance salaries as high as $200 a month are offered, with prospect of promotion in some branches to $250, $300, and higher rates for special positions.

Further information and application blanks may be obtained from the secretary of the U. S. civil service board at Boston, New York, Philadelphia, Atlanta, Cincinnati, Chicago, St. Paul, St. Louis, New Orleans, Seattle or San Francisco, or from the U. S. Civil Service Commission at Washington, D. C.